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SCIENCE: A Weekly Journal, since 1900 the official organ of the American Association for the Advancement of Science. Published by the American Association for the Advancement of Science every Friday at Lancaster, Pennsylvania.

Editors: JOSEPHINE OWEN CATTELL and JAQUES CATTELL.

Policy Committee: MALCOLM H. SOULE, ROGER ADAMS and WALTER R. MILES.

Advertising Manager: THEO. J. CHRISTENSEN.

Communications relative to articles offered for publication should be addressed to Editors of Science, 34 Gramercy Park, New York 3, N. Y.

Communications relative to advertising should be addressed to THEO. CHRISTENSEN, Advertising Manager, Smithsonian Institution Building, Washington 25, D. C.

Communications relative to membership in the Association and to all matters of business of the Association should be addressed to the Permanent Secretary, A.A.A.S., Smithsonian Institution Building, Washington 25, D. C.

Annual subscription, $6.00 Single copies, 15 cents

MICROBIAL METABOLISM AND AGRICULTURE[1]

By Dr. R. E. BUCHANAN

IOWA STATE COLLEGE

THIS paper is designed to point out certain relationships between fundamental studies on microbial metabolism and the great basic science and art of agriculture, and that these advances in physiology, enzymology and physiological chemistry are significant not only in terms of human nutrition and human physiology and curative and preventive medicine, but also in farm production.

Obviously I am not called upon on an occasion of this kind to explore to the limits all the ramose diverticula of modern findings and theories relative to the uptake, synthesis and breakdown of substances useful or harmful to the microbial cell. Rather I should indicate those studies and advances which seem particularly promising in their application to non-microbial organisms. There is a great practical significance to the present and mounting knowledge on microbial metabolism.

Studies on microbial metabolism have stimulated a greater appreciation of the real unity of the basic characteristics of all protoplasm and the wide-spread uniformity of many of its activities. First an example: Until comparatively recently the ability of plants to fix carbon dioxide was conceived to be quite dependent upon the presence of chlorophyll. Then Winogradsky and others found that certain microbes were able to fix carbon dioxide without chlorophyll, and so living things became segregated into those which could not use carbon dioxide and those which could—the latter being subdivided into those which required chlorophyll and light (the photosynthetic forms) and those which utilized reduced inorganic

[1] Address of the retiring vice-president of Section O—Agriculture, American Association for the Advancement of Science, Cleveland, Ohio, September 14, 1944.

compounds such as hydrogen sulfide or ammonia as hydrogen donors in the reduction of the carbon dioxide. Then came the brilliant work of Gaffron, van Niel and others, which revealed that some kinds of organic compounds may replace inorganic when used by certain so-called chemosynthetic bacteria. And then it was found that the exact period during which a green plant was illuminated did not necessarily precisely coincide with the period during which carbon dioxide was being fixed. And then the pioneer work of Werkman and Wood and their colleagues showed that certain bacteria, in the first instance the propionic acid bacteria, can and do fix carbon dioxide, even though they are included in the heterotrophic group. This was revolutionary thinking, quite against the traditions of the texts. But these men by use of the isotopic techniques not only confirmed their earlier findings, but showed the channel through which in this case the carbon dioxide enters into and becomes a part of an organic molecule. Then it seemed that we might recognize three groups of carbon dioxide-fixing organisms, the photosynthetic, the chemosynthetic, certain heterotrophs. But what about the animal kingdom which is entirely heterotrophic? Soon came the evidence that animal tissues likewise may assimilate carbon dioxide, even the tissues of the human body. Through this sequence of studies we have a most surprising and emphatic demonstration of wide-spread uniformities in metabolism of plants and animals, of the essential unity of protoplasm. Studies on bacterial metabolism are bringing us to an understanding of how the corn plant manufactures starch. True, one channel or method only whereby carbon dioxide gets into an organic molecule has been demonstrated for certain bacteria. But it seems improbable that the one first discovered is either the only or necessarily the most important channel or mechanism of carbon dioxide fixation by plants. When we look back upon the development of our knowledge in this field we realize that a part of our difficulty has been slowness to recognize the applicability of certain principles of physical chemistry, such as the reversibility of chemical reactions. May I again emphasize that studies in bacterial metabolism, studies on forms of life possessing no photosynthetic powers, are giving answers to phenomena which are fundamentally basic to agriculture. The apparent ease and facility with which the agricultural and forestry crops manufacture carbohydrates from carbon dioxide and water has irritated and baffled the plant physiologist and the agricultural scientist. Apparently studies on microbial metabolism are furnishing needed keys and tools.

We are woefully deficient in our knowledge of certain phases of nitrogen metabolism of living cells. One of the most puzzling problems since the beginnings of modern chemistry has been the fact that although nitrogen is an essential element in all living protoplasma, and though elementary nitrogen is almost omnipresent, relatively few cells seem able to bring about its fixation and utilization. Nitrogen fixation is one of the really fundamental problems of agriculture. We know that certain free-living soil bacteria and algae can fix nitrogen and build it into protoplasm. We know too that certain bacteria and perhaps other micro-organisms in symbiosis with higher plants can also fix nitrogen. Upon these two facts, so far as our present knowledge goes, we base our whole theory of native soil fertility. The Haber process of nitrogen fixation and its later developments have conceivably made possible an agriculture freed from a base of microbial nitrogen fixation. But far more nitrogen is fixed microbially now, and probably will continue to be, than will be fixed by artificial means. One hesitates to predict, but this situation will probably continue until we have learned just how the microbe fixes the nitrogen. Progress is being made. The work of Virtanen and of Wilson and their collaborators has yielded and is yielding results. It is probable that presently the technique of fixation and the nature of the initial compound or compounds will be clear. What this may mean for practical agriculture is not entirely certain. But we shall know a mechanism which has long baffled us and which may clear up the long-term enigma of the nitrogen-fixing powers of the *Leguminosae* and of some other groups of higher plants.

The chemical changes which micro-organisms bring about in their environment are of real significance in agriculture as well as in other fields. Our knowledge of the steps which are required and of the tools which are used by the organism is gradually being unraveled. We live in a welter of cycles named after this and the other individual; chains of chemical transformations which have been suggested as hypotheses. And each comes nearer to giving us a clear picture of what the cell is doing, what tools it uses and from whence the energy is derived to keep the machine functioning.

The microbial cell has many advantages in the study of certain physiological processes over the cells of multicellular forms. One may have uniform cells, cells at various growth stages, with or without chlorophyll, and with an astonishing gamut of uniformities with which to work. The extension to study and confirmation in physiology of higher plants is proving most useful in an understanding both of normal and abnormal cell physiology.

Micro-organisms have long been used as food, but their production as crop plants has only recently been initiated and somewhat developed. The most outstanding and consistent of the physiological differences between animals and plants in general is the lack of ability on the part of animals to utilize nitrogen

in a form simpler than an amino acid, or at least an amine. Most plants on the other hand can utilize ammonia and even nitrates and other simple nitrogen compounds. From the standpoint of agriculture, in most of our agricultural economies, there is an overbalance of carbohydrate feeds as contrasted with protein, particularly in production of meat, milk and eggs. It is true we may apply the nitrogen to the soil and get crop increases, but with still some inbalance. In recent years there has come the thought that it might be practicable to convert the excess carbohydrates, lignins, etc., into proteins through the intermediation of micro-organisms. Several examples of this tendency may be cited. Previous to the outbreak of this war, a considerable amount of German literature appeared on the subject of developing supplementary protein feeds from cheap carbohydrates and synthetic nitrogen. The fact was established that the yeast cells contained in brewers-grains, distillery slops, etc., make a distinct contribution to the protein value of these as feeds for domestic animals and birds. By shift of emphasis, cheap carbohydrates were sought on which to grow yeasts primarily for their nutritive value. Wood waste, sawdust, was hydrolyzed, the syrup extracted, synthetic nitrogen (ammonia) added and yeasts grown under appropriate conditions of aeration. A reasonably complete conversion of the nitrogen into protein useful for feeding hogs, poultry and other domestic animals resulted. This, of course, in the interest of a self-sufficient economy.

More recently we have been told of the possibility of utilizing the yeast as a human food, not simply as a supplier of certain vitamins, but, as the newspapers had it, in the form of synthetic beef steaks. Work of the Lindegrens in hybridizing the yeasts to get better varieties may well be significant.

But yeasts constitute a tiny fraction of the range of micro-organisms and higher fungi that can attack carbohydrates, even lignins, and which can utilize ammonia with consequent production of proteins. Some development is possible in our post-war world in the utilization of these materials together with our excess production of synthetic nitrogen in the manufacture of cattle and hog feed. Xylans are available in abundance; the production of xylose syrups is feasible. Micro-organisms and fungi are known which can utilize one or the other or both. Great quantities of coarse fodders, cull potatoes, etc., might with some clever planning be converted into high protein feeds, into protein of good quality. We know comparatively little about the fundamental metabolism of most micro-organisms and fungi. But there is little doubt but what a fungus agriculture might put to good use a great amount of present waste and cull material.

Recently we have become increasingly aware that the microbial flora already present or which may be developed in the body of certain animals may be distinctly useful. We now know that urea can at least in part replace proteins when fed to certain ruminants, including the dairy cow. Apparently there exists in the paunch of these animals a microbial flora which utilizes on the one hand the carbohydrates of the food but metabolizes also the urea into the protein of its own cells. Probably the organisms are bacteria. But just how the bacterial protein is utilized by the animal we are not quite sure. Much work is needed by a combination of bacteriologists, protozoologists and physiologists. We know that not only does the paunch contain a rich flora of bacteria but an unusual and startling fauna of protozoa. The recent success of Hungate[2] in cultivating a *Eudiplodinium* from the paunch of cattle is a challenge to further work. These obligately anaerobic ciliates were cultured for 22 months in a medium containing inorganic salts and pulverized dried grass and cellulose. This author shows that not only does this protozoan digest cellulose and contain the enzyme cellulase, but that the synthesis by protozoa of proteins in the paunch supplies perhaps one fifth of the protein requirements of the animal. Here we have an intricate symbiotic mixture of micro-organisms, protozoa feeding on bacteria and cellulose; and the cow utilizing both as protein food. Here certainly we will find eventually close relationships between microbial metabolism and agriculture.

Brilliant work is being done on the characterization of foods and feeds on the basis of the adequacy of their proteins, their amino-acid makeup and their association with vitamins and accessory growth substances. Agriculture benefits from both sides: it produces the food, and it uses the feeds. Analyses for amino-acid content and particularly for vitamins and other accessory growth substances are increasingly important. Here again microbial metabolism is playing an important role. Many of the bacteria are able to synthesize all the various growth accessory substances which they require. Some produce vitamins to such a degree as to be significant when used as foods or feeds. but many others are "born" deficient in ability to produce certain of these. This is particularly true of species that have become highly adapted to a particular environment. Such are to be found among the bacteria parasitic on plants and animals, and others which grow normally on fermenting plant materials. The environment has supplied their need for auxins or vitamins, etc. We find them difficult in consequence to culture, particularly on a synthetic medium. It was not at first clearly recognized that certain of these growth accelerants, etc., required by these organisms were identical with the vitamins. And now we find that these organisms are becoming most useful

[2] *Biol. Bull.*, 83(3): 303–319, 1942.

tools in evaluating the vitamin content of foods. And the same is true for the amino acids. Some organisms can synthesize from the simplest materials all the amino acids which they need. Others can not. There is good evidence that such organisms lack some essential part of the enzyme system necessary for the synthesis of some particular amino acid or even several amino acids. Unless these are supplied ready manufactured, the cell can not grow. And, other conditions being favorable and standard, the rate of increase in the microbial cells is an easily ascertainable mathematical function of the amino-acid content of the medium. The rate of growth becomes a tool for determining quantitatively the amino acid.

Our armamentarium for this purpose has been enriched from two principal sources, the isolation of organisms from special environments and the development of mutations. The latter has been most extensively studied and developed by Beadle for the fungus *Neurospora*. Various mutations have been developed which reveal certain enzyme deficiencies. In some cases fruiting is inhibited by lack of certain substances in the medium. It is evident that those scientific laboratories which service agriculture have now a new array of tools; biology has come to the aid of chemistry in much the same fashion as in the past chemistry has tooled biology. Facility in determination of the quality of foods and feeds will have its abundant fruition in better nutrition for man and animal.

For decades there has been accumulating a considerable microbial literature on the general topic of antibiosis and of staling substances. Observation has long shown that certain types of organisms grow better together, but in other cases one organism may be antagonistic to another. It is common observation that when growing upon a culture medium mold hyphae show either positive or negative chemotropisms. A microscopic view of the margin of certain mold colonies shows that the branches of the filaments as they originate quite accurately bisect the angle between the adjacent hyphae. Evidently the young hyphae grow in the direction of the lowest concentration of excretory products, or conversely show a negative chemotropism with reference to the excretory products of the plant itself. When two colonies approach each other in growth, they are mutually inhibited and do not grow together; *i.e.*, they remain separated. Then, too, one species may inhibit the growth of another. The medium on which an organism has been grown may be positively inhibitory for another. The pyocyanase produced by *Pseudomonas aeruginosa*, actively lytic for certain other bacteria, has long been proposed as a therapeutic agent. Recently major attention has been drawn to these staling or antibiotic or bacteriostatic or bacteriolytic substances when it has been found that some were far more toxic or inhibitive to certain pathogenic organisms than they are to the tissues of the animal body, and we had the discovery and development of penicillin and the various other microbial bacteriostatic or bacteriotoxic substances. And more recently we are gaining an insight into what essential segments of the enzyme systems are put out of commission. We have been so impressed by the efficiency of penicillin in human therapy that we may overlook the agricultural significance of future developments of this type in study on microbial metabolism. The utilization of therapeutic agents in veterinary medicine will follow the regular course of human medicine. But there is a wide-open field in the control of plant diseases, particularly seedling diseases, damping-off, etc. We in agriculture are coming to a realization that fungi attacking roots and seedlings play a great role in limiting crop production. Certain of the pythiaceous fungi are particularly significant. When grown on culture media, inhibition by various fungi is readily observed. A whole field of phytic therapeutics is opening up. The antagonisms and interactions of the fungi and the bacteria make possible the production, isolation and identification of antibiotics, some of which may be useful, and at least will give us clues to work with in our efforts to develop synthetic plant treatments and to explore more adequately the relationships of chemical structure to therapeutic efficiency and toxicity.

Another type of advance which we in agriculture view with great interest is the progress that is being made in determination of the paths of microbial synthesis. I remember years ago—now nearly four decades ago—hearing Dr. Nef, then head of chemistry at the University of Chicago, predict that largely as a result of the analytic studies underway, adequate techniques of synthesis of the carbohydrates would be commercially feasible within a decade. But it has not yet been accomplished. The problem of synthesis is far more complex than originally surmised. But we are making progress. By use of enzyme preparations we are able to carry on these syntheses apart from the living cell. Witness the recent success of Barker and his collaborators in making use of an invertase-free sucrose-phosphorylase from the bacterium *Pseudomonas saccharophila* in the synthesis of sucrose from glucose-l-phosphate and fructose. We are beginning to see how old Mother Nature is able with little apparent effort to synthesize the complex compounds which she produces so abundantly in every green plant. And our microbial studies are increasingly significant.

Knowledge of microbial metabolism is bound eventually to crack the great mystery of virulence in disease production. We know rather accurately what changes certain bacteria produce upon their environ-

ment *in vitro*, but our efforts to correlate with what happens *in vivo* have not been particularly successful. One of our difficulties has been that in most cases we could not readily determine degrees of virulence as contrasted with degree of resistance or immunity. Recently it seems that we are on the track of techniques which should help in unraveling the difficulty. We have learned that measurement of virulence of an organism by the determination of the size of the lethal dose is not entirely satisfactory. Some studies underway on maize show one advantageous technique. Inbred strains of maize become highly homozygous, and the individuals of each strain react with a high degree of uniformity. And by choice of these inbreds one may secure a regular gamut of resistance to certain diseases. Particularly interesting is that caused by *Pseudomonas stewartii*, the Stewart's disease of corn. Some strains of corn are highly resistant to infection, others very susceptible. Furthermore, the causal organism, Stewart's bacterium, can be caused to mutate, and one may secure strains varying widely in attacking power of virulence. We have fixed standards to measure virulence; we have strains which mutate when grown in culture media. But we need vastly more knowledge of the slight metabolic differences among these microbial strains. Variations in virulence presumably must have a basis and be completely correlated with variations in the microbial metabolism. We are gaining command of the tools which are needed to detect these changes. When once we know the metabolic differences among these strains, we shall be for the first time in position to know what constitutes immunity. In this whole series of studies, agriculture will have much of profit.

Agriculture has tremendous interest in the changes which may be effected by micro-organisms in their chemical environment. The studies on basic microbial metabolism are yielding the keys to understanding of the exact chain or sequence of events in the great maze of fermentations and products of microbial change. Higher plants produce vast stores of polymers based upon the hexoses, and upon these most of our present fermentation industries are based. But plants also produce vast stores of polymers based upon the pentoses as well. Pentose sugars could easily be made available in large amounts if we knew satisfactory fermentative uses for them. The sugar xylose is readily secured from many agricultural products. It is not utilized by our domestic animals. It can be attacked by certain micro-organisms. But we need more and better basic information on microbial metabolism before we can satisfactorily unlock effective utilization.

Let's look for a moment at the industries and potentialities based largely upon the use of hexose sugar.

Alcoholic fermentation has long been studied intensively. Pasteur showed the relationship of the yeast to the process; Harden started our modern development of knowledge relative to the enzyme systems in all their complexities. While there is much still to be done, it probably is safe to say that more is known relative to the changes from sucrose or starch to alcohol and carbon dioxide than to those inherent in any other metabolic series. In many respects the studies on this sequence of changes have been basic to our understanding of many other intracellular types of changes. In these studies agriculture has a tremendous stake.

Before exploring this phase of fermentation further, let's look for a minute at the sources from which we may secure the carbon for the multitudinous organic compounds with which civilized man is increasingly concerned. We live in an ocean of air-containing carbon dioxide, and we have access to the oxidized carbon given off in flue gases, and to that found in the great deposits of carbonates in the earth. Carbon dioxide itself is, of course, increasingly useful to man as such. But carbon in most cases is in reduced carbon compounds in which there are carbon-to-carbon linkages, or in which there is hydrogen or hydroyxl, etc. Technology has methods of reducing the carbon of carbon dioxide, but for all practical purposes the organic compounds of modern life have their origin in the growth of plants. The marvelous precision with which plant life takes up carbon dioxide and reduces it and combines it has proved thus far to be our cheapest and most satisfactory technique. True, we may start with coal or with petroleum, but these are but the fossilized products of plant growth. A major economic concern of agriculture is just this: What are the relative efficiencies in securing the organic compounds needed by civilization from coal, from petroleum and from the products of farm and forest? There are broad areas in which each has an advantage. In petroleum and in coal there are already present many useful compounds which only require extraction; many of the products of agriculture, particularly for use as food and feed, have not been and probably can not be successfully duplicated. But there are considerable areas where relative advantages of crops, coal and petroleum are not so clear. For example, one may produce alcohol synthetically from coal, from petroleum, from agricultural products or by fermentation from agricultural products. Concretely at present it breaks down into competition between fermentation alcohol or synthetic alcohol from coal and petroleum. For some decades organic chemistry was coal tar chemistry and ring compound chemistry, and more recently it has been petroleum chemistry and straight chain chemistry. You will recall the ballyhoo two years ago about how our synthetic rubber problems would be promptly settled by

petroleum. The paper chemistry of butane or ethylene to butadiene to rubber is beautiful to behold. But American tanks and guns and jeeps are in action all over the world because yeasts made alcohol from sugar and chemists made this fermentation alcohol into butadiene and this into synthetic rubber. It is true that in this great crisis in motorization and mechanization it was the zymotechnologist and not the petroleum chemist that made rubber supplies possible and reasonably adequate. May I repeat my point. There are many areas in which needed organic compounds can be produced either by fermentation, *i.e.*, by microbial activity or by chemical synthesis. The calm assumption of some of our chemist friends that huge retorts and distillation devices and catalysts and high pressure, etc., constitute the answer should be regarded in many cases with some scepticism. Economics must determine what process is most efficient. The fact that quinine has been synthesized at Harvard recently doesn't mean that the Cinchona tree may not be the most satisfactory source of the drug any more than the demonstration of an enzyme system that can combine fructose and glucose into sucrose means that we will quit producing sucrose from the sugar cane in Cuba. I am insisting that a practical chemical economy in the future depends quite as much upon our exploration of the fundamentals of microbial metabolism as upon the exploration of petroleum chemistry, conceding the vast importance of the latter. A substantial part of the economics of agriculture, of the future of agriculture, is bound up in the adequate exploration of microbial metabolism.

Fermentation products frequently have an advantage in that they are relatively pure, or may be readily purified. Commonly they are not mixed with isomers. For example, 2-3 butylene glycol is a product which can be produced commercially by fermentation with facility. But there are several isomers of this compound which show interesting difference in behavior. One apparently would make a good anti-freeze for your automobile radiator, the others will not. Chemical synthesis is apt to yield a mixture with great difficulty in separation. But certain bacteria produce the desired isomers practically pure.

The stake of agriculture in the problems of chemical utilization are considerable and material. Right now millions of bushels of grain are being converted into rubber. To what extent may agriculture look to fermentation industries in the future to assist in relieving us of great surpluses of grains, surpluses which are absolutely inevitable without major changes in world economy, changes which at the best can be brought about but slowly? Crop residues likewise must be studied directly as competitors of coal and petroleum in the future development of our agricultural economy. Fermentation products will increasingly constitute the raw materials for chemical synthesis and transformations.

In summary, microbial metabolism, microbial nutrition, real continuance of research in fundamental fields relative to the physiology of micro-organisms, are of quite as much significance to agriculture as to medicine or to biology as a whole. We are on the verge of new advances which may have the most far-reaching consequences to standards of living and to civilization.

OBITUARY

ROBERT BENNETT BEAN
1874–1944

ONE of America's outstanding anthropologists and teachers of anatomy, Dr. R. Bennett Bean, died on August 27, 1944, in Staunton, Virginia. He had resigned from his position as head of the department of anatomy in the University of Virginia School of Medicine two years previously because of ill health brought on by arteriosclerosis. His death was not unexpected but was a source of grief to many friends, associates and former students as well as his family.

Dr. Bean was born at "Pleasant Hill," Gala, Botetourt County, Virginia, on March 24, 1874, the third of a family of eight children born to William Bennett and Arrianna Williamson Carper Bean, whose ancestors had been early colonists of Virginia and Maryland, respectively. His father had carried on a hectic courtship between campaigns with the Army of Northern Virginia in which he served as a lieutenant in the Baltimore Light Artillery. Dr. Bean's boyhood and youth were spent under the most trying circumstances of economic distress in the backwash of the Reconstruction period following the war between the states. His formal schooling was intrusted to an aunt, Miss Regina Bean, whose teaching skill and discipline made a lasting impression. Despite the rigors of the times his home life was unusually happy and members of the large family were loyal and devoted. The Bible was a strong factor in his education and he retained deep religious convictions throughout his life.

From the age of thirteen until he entered the Virginia Polytechnic Institute at the age of twenty-two there was no opportunity for formal schooling. Experiences in farm work, clerking in a county store, managing the dining room at the old summer resort at Daggar's Springs, teaching school, mining, guarding convict labor, selling farm machinery and writing for a county newspaper gave a wide background of use-

ful knowledge. Besides working his way through college with a high scholastic record he engaged in football and track; and retained a lifelong interest in sports. In 1900 he received his B.S. degree from Virginia Polytechnic Institute and was enabled to enter medical school through the generosity of his sister, Miss Mary Cloud Bean. He studied at the Johns Hopkins Medical School under Osler, Mall, Welch, Kelly, Halsted and others. Dr. Bean was deeply imbued with the spirit of research and teaching, and was especially influenced by Mall and Osler. He began his career as an anatomist and anthropologist during his student days. Following graduation in the class of 1904 he remained in Mall's laboratory as assistant in anatomy, pursuing studies of the subclavian artery and racial peculiarities of the Negro brain. In 1905 he became assistant professor of anatomy at the University of Michigan. During the summer of 1906 he studied in the Ecolé d'Anthropologie de Paris under Manouvrier, whose anthropometric techniques and high standards of accuracy he emulated. From 1907 to 1910 Dr. Bean was the director of the Anatomical Laboratory of the University of the Philippines; made extensive observations on physical anthropology of Filipino tribes; and obtained many specimens for the Wistar and Smithsonian collections. Returning to this country he served as associate and professor of anatomy at Tulane. In 1916 he became head of the department of anatomy at Virginia.

Dr. Bean's career was a happy combination of teaching and research. Having decided on anatomy as a life work his investigations in anthropology were a natural development—carrying the study of man outward to the macrocosm of type and race rather than inward to microanatomy and the biophysics of the cell. He had a highly original mind and made several notable contributions in his selected field. Early studies led him to associate particular ear structure and physical type of man. In 1909 he published his first work on morbidity and morphology based on autopsy material, demonstrating an association between body type and predilection to different diseases. Though the belief that type might be related to disease is as old as Hippocrates this was the first objective demonstration of its truth. Several subsequent articles extended and further validated his original thesis, which is now accepted as axiomatic. Another study culminated in a report on "The Pulse of Growth," emphasizing alternate periods of slow and rapid development of tissues and organs from embryonic life to maturity. Other studies included characterizing the physical anthropology of Filipinos; the so-called Mongolian idiot; and old Virginians. He published 72 papers and three books: "Racial Anatomy of the Philippine Islanders," "The Races of Man" and "The Peopling of Virginia."

His excellence as a teacher of anatomy will be remembered by several generations of students. He made a model of the peritoneum which clarified much of its inherent complexity; and taught cerebral nerves in a logical rather than traditional manner. He believed that dissection and demonstration were the prime tools in learning anatomy. Text-books and models were complementary to dissections and lectures. His informal lecture, the quiz-talk, was highly successful though not without some terror for the lazy student. Much of its value lay in the charm and dignity of his personality and the high ideals of scholarship and research he taught and exemplified. His relationship with his students is epitomized in his nickname—"The Baron." Anecdotes were freely used to bring home a point, and the aphorisms and teachings of Sir Thomas Browne, Osler, Pasteur, Darwin, Hunter and others were mingled with his own. A host of students recall the "area of abdominal romance" where the head of the pancreas lies in the arms of the duodenum and has its feet tickled by the spleen when they have forgotten more erudite aspects of anatomy. His essay on teaching anatomy might be read with profit by many to-day.

Though his chief contribution was as teacher and investigator he was active in other ways. When funds became available to erect new buildings for the Medical School at the University of Virginia he was made chairman of the building committee.

In 1907 Dr. Bean married Adelaide Leiper Martin. Their life together was singularly happy. In addition to his widow he is survived by four children, Mrs. James Van Duesen Eppes, Major William Bennett Bean, M.C., AUS, Mrs. Natt Morrill Emery and Chaplain George Martin Bean, U.S.N.R. At one time or another they have all been his collaborators and served an apprenticeship at least in the lesser mysteries of the slide rule. The custom of reading aloud to the family made a strong impression on all its members.

Dr. Bean's rank as a scientist was recognized by his peers and is attested to by membership in numerous scientific societies. He was first president of the Virginia chapter of Sigma Xi, which he was instrumental in establishing. In 1914 he was president of the New Orleans Academy of Science; in 1928 he was president of the Anthropological Section of the American Association for the Advancement of Science. For many years he was an associate editor of the *American Journal of Physical Anthropology*. He was a member of the Association of American Anatomists, the American Association of Physical Anthropologists and the Societa Romana Anthropologica.

The strong currents of religious conviction and scientific scholarship never caused any perceptible conflict in his life. The Bible on one hand and the rigor-

ous disciplines of research exemplified by Darwin, Pasteur, Mall, Manouvrier and Hrdlička stamped him. He was more interested in the substance of science than the surface trimmings. His writings were straightforward and clear, never polished and never ornate. His guide to English style was Albutt, whose "Notes on the Composition of Scientific Papers" he followed for precept, not example. Taught by a Catholic aunt, a regular attendant at the Presbyterian Church as a youth, Dr. Bean later became a member of the Episcopal Church, of which he was a loyal supporter and vestryman. He had more regard for underlying principles than dogma. An abiding belief in simple Christian virtues characterized his dealings with his fellow man. He was always a friend and often a counselor of his students. His generosity and anonymous benefactions in the community still come to light from time to time. During a period when moral and scientific standards have often given place to laxity and opportunism, he remained true to the highest ideals; and his teachings and example were such that memory of him is a reaffirmation of faith in human dignity and honor.

WILLIAM BENNETT BEAN,
Major, M.C., AUS

RECENT DEATHS

DR. ROBERT K. BREWER, head of the department of physiological chemistry of the College of Medicine of Syracuse University, died on March 22.

DR. HENRY LANE BRUNER, professor emeritus of biology and geology of Butler University, with which he had been connected for forty-six years, died on March 17 at the age of eighty-four years.

DR. GEORGE W. HUTCHISON, since 1933 secretary of the National Geographic Society, died on March 24 at the age of fifty-eight years.

ROBERT HALLOWELL RICHARDS, professor emeritus of mining engineering and metallurgy at the Massachusetts Institute of Technology, died on March 27. He was one hundred years old.

DR. LELAND RUSSELL VAN WERT, consulting metallurgist and chief of the metallurgical division of the Leeds and Northrup Company, Philadelphia, makers of electrical measuring instruments, died on March 27 at the age of fifty-five years.

DR. NEVIL MONROE HOPKINS, consulting and research engineer, died on March 26. He was seventy-one years old.

SCIENTIFIC EVENTS

THE BRITISH THERAPEUTIC TRIALS COMMITTEE[1]

DR. F. H. K. GREEN, of the administrative staff of the Medical Research Council, describes in the *British Medical Bulletin* the work of that council's Therapeutic Trials Committee. In response to representations by the Association of Chemical Manufacturers, the Medical Research Council organized in 1931 a scheme for the clinical testing of new remedies, and the Therapeutic Trials Committee was set up as a disinterested intermediary between the manufacturers and the medical profession, some medical men having been reluctant to carry out tests at the request of commercial firms. It was agreed that foreign as well as British remedies should be tried out and also the products of academic as well as of commercial laboratories. Manufacturers desiring trials by the Medical Research Council must agree to certain conditions. The composition and nature of the substance to be tested must be fully revealed to the council; manufacturers must not, without the council's permission, arrange for other independent trials, and the council is interested only in new substances which have not been therapeutically tested.

When a substance is to be tested, arrangements are made with clinicians of high standing to make tests, usually at more than one hospital, and the council reserves the right to decide whether the results, favorable or not, shall be published or revealed only to the manufacturer. If a clinician's results are published, they are published under the clinician's name as a report to the Therapeutic Trials Committee. Since the scheme was organized in 1931, more than forty new substances have been tested clinically. Outstanding examples are the classical papers embodying the results of clinical trials of "prontosil rubrum," which established the therapeutic possibilities in man of the first sulphonamide drug, which had been discovered in Germany; some of the earliest controlled clinical tests of sulphanilamide; trials of stilbestrol and other synthetic estrogenic agents. During the war clinical tests of penicillin have been organized and are still going on, and British-made equivalents of important foreign pharmaceutical products are being tested. The control of infections of wounds and burns is also being studied. Ultimately, according to Dr. Green, it is at the bedside that the clinical value of any new remedy is decided.

[1] From *Nature*.

MEMORIAL LOAN FUND FOR GRADUATE STUDENTS IN SCIENCE

As a memorial to the late Professor H. H. Whetzel, his former students and associates in the Department

of Plant Pathology at Cornell University are raising a graduate student loan fund to be administered by the university. Professor Whetzel, who was always much interested in the welfare of the graduate student in science, was concerned over the inadequacy of funds for loans, and gave considerable time and thought to ways of correcting the situation. In more recent months before his death he was especially concerned with the problem of depleted ranks in both undergraduate and graduate students, as the result of the war, and saw in a graduate student loan fund the means of helping some promising students who otherwise could not finance their graduate work. He did not live to see any of his plans for obtaining money materialize, but it is believed that the Memorial Loan Fund may serve to fulfil his purpose in part. While solicitations are being restricted largely to former students and associates, and close friends, contributions from any one desiring to assist will be most welcome. Further information may be had from Professor L. M. Massey, of the Department of Plant Pathology, College of Agriculture, Cornell University, Ithaca, N. Y.

THE CLEVELAND PHYSICS SOCIETY

PHYSICISTS, meeting at the Case School of Applied Science, have formed the Cleveland Physics Society, which is intended to serve the academic and industrial physicists of Greater Cleveland in a manner similar to that in which the engineering societies serve the engineers.

Over one hundred members attended the meeting (March 22) which was addressed by Professor P. M. Morse, of the department of physics of the Massachusetts Institute of Technology. His topic was "Recent Developments in Room and Auditorium Acoustics."

Officers elected were as follows: *President,* Leonard O. Olsen, assistant professor of physics, Case School of Applied Science; *Vice-president,* Clifton G. Found, director of physical research, General Electric Company, Nela Park; *Secretary,* John T. McCarthy, assistant professor of physics, Western Reserve University; *Treasurer,* W. Byron Brown, physicist, N.A.C.A. Engine Laboratory. Elmer Hutchisson, dean of the faculty of the Case School, was elected a member of the executive council.

It is planned to hold regular monthly meetings which will be addressed by prominent physicists who will speak on subjects of rather general interest. In addition informal seminars will be held occasionally on more specialized subjects.

Information concerning meetings or membership in the society may be obtained from the secretary, Professor John T. McCarthy, at Western Reserve University by mail or telephone.

MEDALS OF THE FRANKLIN INSTITUTE

IN addition to the Medals of the Franklin Institute announced in SCIENCE last week the Franklin Medal has been awarded to Dr. Harlow Shapley, director of the Harvard College Observatory, "in consideration of his many valuable contributions to the science of astronomy, and especially of his work in the measurement of the vast distances necessary for the determination of the nature and extent of our galaxy, as well as those of other galaxies external to ours."

The Potts Gold Medal for 1945 has been awarded to Edwin A. Link in recognition of "valuable contributions in the field of training devices for aviators." The medal was established in 1906 by the bequest of Howard N. Potts to be given for distinguished work in science or the mechanic arts.

The Edward Longstreth Medal has been awarded to Sanford Lockwood Cluett, vice-president of Cluett, Peabody and Company, Troy, N. Y., "in view of the fundamental nature and mechanical ingenuity displayed in the development of the process for the preshrinking of woven fabrics, known as Sanforizing."

The Louis E. Levy Medal has been awarded to Dr. Rupen Eksergian, consulting engineer for the Edward G. Budd Manufacturing Company, Philadelphia, for his paper entitled "On the Reaction of Fluids and Fluid Jets," which appeared in the issue of the *Journal* of the institute for May, 1944.

SCIENTIFIC NOTES AND NEWS

THE Washington section of the American Chemical Society presented on March 8 the Hillebrand Prize for 1944 for an outstanding contribution to the science of chemistry during the three preceding years to Raymond M. Hann, of the National Institute of Health, in recognition of his work on the chemistry, structure and synthesis of methylene and benzylidene acetals of sugar alcohols.

AT the thirty-second annual meeting in Chicago of the American Social Hygiene Association, the William Freeman Snow Medal for distinguished service to humanity was presented to Major General Merritte W. Ireland, formerly surgeon general of the U. S. Army.

AT the seventh annual Forum on Allergy, meeting at Pittsburgh, the first Marcelle award was conferred on Dr. Mary Hewitt Loveless, of the New York Hospital, and the second award was presented to Dr. Charles F. Code, of the Mayo Foundation, Rochester, Minn.

THE first Sidney Powers Memorial award for outstanding contributions to petroleum geology was presented on March 27 at the recent annual meeting of the American Association of Petroleum Geologists to Wallace Pratt, a director of the Standard Oil Company of New Jersey.

THE American Association of University Women has conferred the annual achievement award of $2,500 on Dr. Katharine B. Blodgett, of the General Electric Research Laboratory at Schenectady, N. Y.

THE Buchan Prize for 1945 of the Royal Meteorological Society, London, has been awarded to E. L. Hawke, secretary of the society.

Nature reports that on February 2, at the Royal Botanic Garden, Edinburgh, Sir William Wright Smith, King's Botanist in Scotland, regius keeper of the Royal Garden and professor of botany in the University of Edinburgh, was presented with a portrait of himself on the occasion of his seventieth birthday. Sir John Stirling-Maxwell presided, and the presentation was made by the Earl of Stair. At the same ceremony a second portrait of Sir William was presented to the Botanic Garden by Sir John Fraser. The portraits, which were painted by Stanley Cursiter, were the gift of a number of Sir William's botanical and horticultural colleagues and other friends.

A SMALL group of friends of Dr. Albert Kenrick Fisher on the evening of March 21 gave him a surprise dinner at the Cosmos Club in Washington in celebration of his eighty-ninth birthday. Until his retirement, Dr. Fisher was for forty-six years a member of the staff of the Bureau of Biological Survey, U. S. Department of Agriculture (now the Fish and Wildlife Service, Department of the Interior), where he carried on natural-history investigations for the Government and organized the Federal program of predatory-animal and rodent control. He was a member of the Death Valley Expedition in 1891, the Harriman Alaska Expedition in 1899 and the Pinchot South Seas Expedition in 1929. He was one of the founders of the American Ornithologists' Union and is the author of "Hawks and Owls of the United States," a pioneer work in economic ornithology published in 1893. On his retirement from active service in 1931, Dr. Fisher was appointed honorary collaborator in biology of the Smithsonian Institution.

DR. ANDREW C. IVY, professor of physiology at the Medical School of Northwestern University, has been elected a member of the National Advisory Cancer Council of the U. S. Public Health Service.

CAPTAIN CURTIS SAUNDERS, Sanitary Corps, Division of Parasitology, Army Medical School, Washington 12, D. C., has been elected a fellow of the Royal Entomological Society, London.

A DINNER in honor of the sixty-fifth birthday anniversary of Howard W. Blakeslee, since 1928 science editor of the Associated Press, was given recently at the Waldorf Astoria Hotel in New York City. The occasion marked the completion of forty years' service with the Associated Press. The speakers included Charles E. Honce, assistant general manager of the Associated Press; Dr. Robert C. Hockett, scientific director of the Sugar Research Foundation, and Mr. Blakeslee.

THE newly elected officers of the Wasmann Biological Society of Loyola University, Chicago, are: *President,* Charles J. Wideman, S.J.; *Vice-president,* Floyd A. Jenkins, S.J.; *Secretary-Treasurer,* J. W. Hudson, all of the university. Edward L. Kessel, of the University of San Francisco, was elected editor.

DR. RALPH PEMBERTON, professor of medicine in the Graduate School of Medicine of the University of Pennsylvania, has been elected president of the Pan-American League for the Study and Control of Rheumatic Diseases.

DR. JACOB A. BENNER, professor of mathematics at Lafayette College, has been appointed director of the summer session.

DR. W. HENRY HOLLINSHEAD, associate professor of anatomy at the School of Medicine of Duke University, will be visiting associate professor of anatomy at the School of Medicine of the University of Utah during the summer quarter.

DR. HELEN WALTER DODSON, since 1943 on leave for war work in the Radiation Laboratory of the Massachusetts Institute of Technology, has been appointed associate professor of astronomy and mathematics for 1945–46 at Goucher College.

LORIN E. HARRIS, formerly agent for the Fish and Wildlife Service, U. S. Department of the Interior, and more recently research associate of the School of Nutrition of Cornell University, has become associate professor of nutrition at the Utah State Agricultural College at Logan.

DR. C. G. C. CHESTERS has been appointed professor of botany at University College, Nottingham, in succession to Professor T. A. Bennet-Clark.

THE American Medical Association has made a grant to Iowa State College in support of research on penicillin, which is being carried out under the direction of Dr. C. H. Werkman. Associated with him in the work are Dr. L. O. Krampitz, professor of bacteriology, and graduate students.

DR. W. B. KEMP, acting director of the Maryland Agricultural Experiment Station, has been appointed

director to succeed Dr. Roger B. Corbett, who has been on leave to work with the American Farm Bureau Federation.

DR. ERWIN H. ACKERKNECHT, a former research fellow at the Institute of the History of Medicine of the Johns Hopkins University, has been appointed assistant curator of physical anthropology of the department of anthropology of the American Museum of Natural History. He will be in charge of exploring and organizing plans for an Institute of Human Morphology at the museum under the direction of Dr. Harry L. Shapiro, chairman of the department of anthropology.

DR. JAMES T. LOWE, special counsel for the Wisconsin Alumni Research Foundation, has been appointed director of nutritional research for Nestlé's Milk Products, Inc.

PROFESSOR F. Y. HENDERSON has been appointed director of Forest Products Research in the British Department of Scientific and Industrial Research on the forthcoming retirement of W. A. Robertson, who has been director since 1933. Professor Henderson is at present assistant professor in timber technology at the University of London and at the Imperial College of Science and Technology. He will take up his new work on April 1.

DR. ARTHUR W. HAUPT, associate professor of botany at the University of California at Los Angeles, has left for Chicago to assume the task of arranging for the publication of the research work of the late Dr. Charles J. Chamberlain, of the University of Chicago. Dr. Chamberlain, who had worked for some thirty years on an exhaustive treatise on the Cycads, a group of tropical plants, died before his study was finished. At the invitation of the University of Chicago, Dr. Haupt will prepare the monograph for publication.

NELSON C. BROWN, head of the Department of Forest Utilization of the New York State College of Forestry, Syracuse University, is in Italy, where he has been appointed forestry consultant with the Allied Control Commission to advise commissioners in the reorganization of Italy's forestry program and survey and make recommendations regarding the country's lumber requirements for reconstruction.

DR. JAKOB BJERKNES, professor of meteorology at the University of California at Los Angeles, has returned to the university after a trip to Guam for the Army Air Forces.

DR. KIRK BRYAN, professor of physiography at Harvard University, spent the winter term in Mexico, on a dam-site consulting project, and in Guatemala, where he carried on geological field work for the Carnegie Institution of Washington.

DR. PEYTON ROUS, of the Rockefeller Institute for Medical Research, will deliver during the period from March 30 to May 9 a series of Sigma Xi lectures on cancer at thirty colleges and universities.

DR. WILLIAM F. WINDLE, professor of neurology at the Medical School of Northwestern University, will deliver on April 19 the seventh Harvey Society Lecture of the current series at the New York Academy of Medicine. He will speak on "Respiratory Conditions in the Fetus and Effects of their Impairment."

DR. FRANZ WEIDENREICH, honorary director of the Cenozoic Research Laboratory, formerly professor of anatomy at Peking Union Medical College, will deliver at the University of California at Berkeley five Hitchcock lectures during April on "The Physical Evolution of Man."

DR. PHILIP B. ARMSTRONG, professor of anatomy at the School of Medicine of Syracuse University, gave the Alpha Omega Alpha Lecture to the faculty and students of the School of Medicine of the University of Georgia on February 21. His subject was "Cholinesterase at Peripheral Nerve Terminations."

DR. JAMES WATSON WHITE, of New York City, on April 6 inaugurates the John O. McReynolds Lectureship in Ophthalmology recently established by Colonel and Mrs. Frank W. Wozencraft, of Washington, D. C., at the Medical Branch at Galveston of the University of Texas.

DR. HARLAN T. STETSON, of the Massachusetts Institute of Technology and director of the Cosmic Research Laboratory, Needham, Mass., on March 14 addressed the students at Wilson College, Chambersburg, Pa., on "Science Tomorrow." On March 15 he lectured at Dickinson College, Carlisle, the subject there being "Our Future in the Sun."

IN the interest of avoiding congestion of hotel and transportation facilities during 1945, the Council of the Institute of Food Technologists has voted unanimously not to petition the Federal Committee on Conventions and Conferences for consent to hold its 1945 conference originally scheduled for Rochester, N. Y., in May. Except for the holding of this conference, the activities of the institute will proceed as usual with the election of officers, the holding of a summer meeting of the council authorized to transact essential business, the holding of regional sections or group meetings and the publishing of its *Proceedings*.

THE forty-seventh annual meeting of the American Ceramic Society will be held at the Hotel Statler, Buffalo, N. Y., on April 16 and 17. Conforming with the Government War Committee directive limiting "out-of-district" meetings to fifty persons, the divisions of the society will forego their usual technical

sessions, and members, other than past-presidents, officers and committee chairmen, are requested not to attend the business meetings. Hotel reservations should be made direct with the Hotel Statler, Buffalo, N. Y.

THE Minnesota Chapter of the American-Soviet Medical Society held a symposium on February 15 on the "Development of the Blood Bank." Professor Maurice B. Visscher, of the department of physiology of the University of Minnesota, presided and introduced the discussion with a review of the work on the utilization of cadaver blood in the U.S.S.R. and the organization of the first blood bank in the United States. Dr. E. B. Flink discussed the problems of transfusion reactions. Speakers included Dr. D. State, Dr. S. A. Corson, Dr. D. A. Needham, Captain J. Mehlman and Lieutenant Commander J. W. Edwards. The proceedings will be published in the next issue of the *Bulletin* of the Minnesota Medical Foundation.

IT is planned to erect at the cost of $1,500,000 a new building for the Medical and Dental Schools of Loyola University, Chicago. Science laboratories, lecture halls, student commons and faculty lounges will be arranged so that they may be used both by the medical and dental schools. It is hoped that the plan will encourage cooperation between the professors of medicine and dentistry.

DISCUSSION

ANAEROBIC RESPIRATION OR FERMENTATION

SEIFRIZ[1] has argued in SCIENCE for the use of the term "respiration" for all forms of energy liberation in living cells and for the retention of the term "anaerobic respiration" for energy liberation in the absence of oxygen. Our attention should be directed to the concepts involved with full recognition that terms are but useful symbols. However, confusion concerning terms may lead to confusion in ideas.

Several patterns of biological energy liberation may be recognized:

(1) Oxidation of organic compounds with the simultaneous reduction of molecular oxygen to water. The oxidation may be complete, with all the carbon converted to CO_2, or it may be incomplete with the formation of organic acids as end products. This type of oxidation is known to most cellular physiologists as respiration or cellular respiration.

(2) Oxidation of inorganic compounds and the reduction of oxygen to water, such as the oxidation of ammonia to nitrites, nitrites to nitrates, etc., in chemosynthetic bacteria. No suitable term for this "inorganic respiration" has been proposed.

(3) Degradation of an organic molecule into two or more simpler molecules by an oxidation and reduction occurring within the original molecules or its products. This is illustrated by the conversion of glycogen or glucose into lactic acid (glycolysis) or into alcohol and CO_2 (alcoholic fermentation), etc. This type of mechanism does not require molecular oxygen, and may or may not be suppressed by molecular oxygen. It is known as fermentation, intramolecular respiration or anaerobic respiration.

(4) Oxidation of organic compounds with the simultaneous reduction of carbon dioxide, nitrates or nitrites, or sulfates. This type of reaction has been particularly studied by Barker.[2] It is included by some under the name of fermentation, but probably needs a distinct term.

There are several features common to all these types of metabolism, and two or more types may occur in the same cell, even simultaneously. We may use the term respiration in a generic sense to include all metabolic processes liberating energy (occurring with a free energy decrease) and have specific names for the different types of respiration, such as aerobic respiration for types 1 and 2, and anaerobic respiration for types 3 and 4, or we may use respiration for metabolism involving oxygen consumption and use fermentation for metabolism of type 3 and perhaps 4.

The writer objects to anaerobic respiration, because it implies a type of metabolism that only occurs in the absence of molecular oxygen. However, it is well recognized that in some tumors, contracting muscles, certain yeasts and some varieties of seeds, the pathway of carbohydrate degradation is fermentative, resulting in the accumulation of lactic acid, ethyl alcohol or other products, even when the tissues are exposed to oxygen. The terms aerobic and anaerobic glycolysis are widely used and aerobic and anaerobic fermentation are in frequent use. If we follow Seifriz we will be forced to discuss anaerobic respiration occurring aerobically!

A second objection to the use of anaerobic respiration is that it has led to a confusion of ideas particularly when the plant physiologist measures metabolism only by the carbon dioxide liberated, for in some cases the rate of carbon dioxide production is the same under aerobic and anaerobic conditions. Such an

[1] William Seifriz, SCIENCE, 101: 88–89, 1945.

[2] H. Albert Barker, *Ann. Rev. Biochem.*, 10: 555–586, 1941.

experimental result led in a manuscript submitted to the *American Journal of Botany* to the following statement, "The respiration was identical under aerobic and anaerobic conditions." In the experimental situation the anaerobic metabolism was almost certainly alcoholic fermentation, and if we examine the two equations we can see the errors in this statement:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$
$$\Delta F = -704 \text{ kg. cal.}$$
$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$
$$\Delta F = -54 \text{ kg. cal.}$$

Since the rate of carbon dioxide production was equal in each case, 3 times as much glucose disappeared under anaerobic conditions, alcohol accumulated, and the rate of energy liberation was but 23 per cent. the aerobic rate. A common term, respiration, led the unwary into calling a difference in energy liberation of over 400 per cent. an identity!

There appears to be objection to the use of fermentation in reference to higher organisms because it has been used uncritically for the metabolism, regardless of its nature, occurring in certain micro-organisms. However, the term has a more precise meaning, as a type of metabolism without reference to the organism in which it occurs. That the later term is justified may be seen from historical considerations. Fermentation initially meant the effervescence of a gas, either in brewing or in a chemical reaction. Pasteur[3] recognized fermentation as a type of energy liberation occurring in certain micro-organisms, but he also recognized that ordinary oxygen consumption could occur in such organisms. Later he[4] broadened the term, fermentation, to include a type of metabolism regardless of the organism in which it was found. On page 267 (*loc. cit.*) he says, "We can even conceive that the fermentative character may belong to every organized form, to every animal and vegetable cell, on the sole condition that the chemico-vital acts of assimilation and excretion must be capable of taking place in that cell for a brief period . . . without the necessity . . . of atmospheric oxygen." On page 273 *et seq.* Pasteur says, "Our theory mentions that all cells become fermentative when their vital action is protracted in the absence of air, which are precisely the conditions that hold in the experiments on fruits immersed in carbon acid gas. The vital energy is not immediately suspended in their cells, . . . consequently, fermentation must result." There is thus excellent justification in the writings of Pasteur for fermentation as applied to higher plants and animals.

Respiration is a very old term in the medical literature, and it meant the inspiration and expiration of air. During the mid-nineteenth century the term was broadened to include the utilization of oxygen in cellular oxidations. This aspect has been well reviewed by Scheer.[5] Pfeffer[6] recognized that anaerobic metabolism occurred in higher plants, and he designated it by intramolecular respiration, though he clearly uses the term as equivalent with fermentation. The writer has been unable to locate the first use of anaerobic respiration, but the term has become widely adopted in our text-books of plant physiology. However, the author does not believe our texts are authorities to be blindly followed when their usage is less precise than that of many research workers in a particular field.

The writer believes we would do well to retain respiration for metabolism of type 1, above, and retain fermentation for type 3. If this is objectionable to some, intramolecular respiration is preferable to anaerobic respiration. Seifriz implies that those who disagree with him (and are unnamed) are not plant physiologists but physiological chemists. I do not see the relevance of Seifriz's classification of scientists to the subject under discussion. Further, Seifriz claims his views are those of plant physiologists, medical physiologists (are they to be denied the use of their term glycolysis?) and bacteriologists. At least one plant physiologist raises his voice in dissent.

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[3] Louis Pasteur, *Ann. de Chimie et de Physique*, 3rd S. 58: 323, 1860.

[4] Louis Pasteur, "Studies on Fermentation, The Diseases of Beer." Eng. ed. London, 1879.

[5] B. F. Scheer, *Ann. Sci.*, 4: 295, 1939.

[6] W. Pfeffer, *Landw. Jahr.*, I: 805, 1878.

"PHOTOPERIODISM" VERSUS "PHOTOPERIODICITY"[1]

BIOLOGISTS who have had occasion to refer to the literature on the influence of the length of the daylight period on living organisms have doubtless noticed that botanists refer to this phenomenon as "photoperiodism," while most zoologists use the term "photoperiodicity." Assuming that such synonymy is useless and confusing and that there is still a possibility of eliminating it in a field as new as this, it may be well to consider the origin, use and aptness of the two terms in an effort to reach some conclusion as to which of the two terms is preferable.

The term "photoperiodism" was not used by Garner and Allard[2] in their 1920 paper in which they announced the discovery of the influence of the length of the daylight period on the growth and reproduction of plants, but was used in a brief paper[3] which appeared in SCIENCE in 1922. In both this paper and the paper entitled "Further Studies in Photo-

[1] Contribution No. 87 from the Science Divisions of the University of Houston.

[2] W. W. Garner and H. A. Allard, *Jour. Agr. Res.*, 18: 553–606, 1920.

[3] W. W. Garner and H. A. Allard, SCIENCE, 55: 582–583, 1922.

periodism"[4] which appeared in 1923 the authors stated that they were "indebted to Mr. O. F. Cook of the Bureau of Plant Industry for suggesting the term photoperiodism, which seems to meet all requirements as to both aptness and simplicity." Following Garner and Allard's lead, other botanists working in this field adopted the term and have used it exclusively in both this country and Great Britain ever since.

The first experimental report on the influence of the photoperiod on animals was that of Marcovitch[5] on plant lice in 1923. However, comprehensive studies of this phenomenon in animals did not begin until 1930, when the first of Bissonnette's numerous papers appeared. Bissonnette was the first to use the term "photoperiodicity." In a letter to the writer he described the origin of the term as follows:

> This term arose when Ross Harrison, editor of the *Journal of Experimental Zoology,* objected to my use of the term "photoperiodism" after Garner and Allard in a paper[6] back about 1930. He said he saw that I had coined a new word "photoperiodism" in place of "photoperiodicity," and I let it go at "photoperiodicity," which now has come to include any periodic or rhythmic process controlled by photoperiods. It is not only reproduction controlled photoperiodically, but includes pelt cycles, plumage cycles in birds and migrations also. So I guess I am responsible for its appearance in biological literature.

Zoologists have in general, but not exclusively, followed Bissonnette in his use of the term photoperiodicity, rather than Rowan,[7] who in 1926 took over Garner and Allard's term. As examples of the exception, Baker in 1936[8] and Rollo in 1941[9] both use the term "photoperiodism."

There seems to be some doubt in Bissonnette's mind as to the strict synonymy of the two terms, as indicated by the above quotation and the following one[10]: "Garner and Allard . . . coined the word 'photoperiodism' for the response of plants to changes in relative lengths of day and night by beginning to bloom, or their exhibition of 'sexual' photoperiodicity." It seems clear, however, from the use of the term by Garner and Allard and other botanists and from the dictionary and encyclopedia definitions, that "photoperiodism" is not a term applied solely to the reproductive aspects of the phenomenon.

As far as the intrinsic merits of the two words are concerned, "photoperiodicity" is probably the better, because "periodicity" is a recognized and widely used term, whereas there is no such word as "periodism." However, it appears to the writer that the term "photoperiodism" should be used by biologists instead of the term "photoperiodicity" for the following reasons: (a) It was the first term proposed. (b) It is much more widely used than the term "photoperiodicity." Not only do some zoologists use it, but all botanists use the term, and to date much more work has been done on plants than on animals in this field. (c) Both the Encyclopedia Britannica and Webster's New International Dictionary list the word "photoperiodism" but fail to list "photoperiodicity," even as a synonym. (d) Although this is perhaps a minor matter, it is somewhat shorter and easier to pronounce than "photoperiodicity."

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[4] W. W. Garner and H. A. Allard, *Jour. Agr. Res.*, 23: 871–919, 1923.
[5] S. Marcovitch, SCIENCE, 58: 537, 1923.
[6] T. H. Bissonnette, *Jour. Exp. Zool.*, 58: 281–319, 1931.
[7] W. Rowan, *Proc. Boston Soc. Nat. Hist.*, 38: 147–189, 1926.
[8] F. C. Baker, *Canadian Entom.*, 67: 149–153, 1936.
[9] M. Rollo, *Bird Banding*, 12: 161–164, 1941.
[10] T. H. Bisonnette, *Quart. Rev. Biol.*, 11: 371–386, 1936.

SPONGE NAMES

IT is to be hoped that there will soon be renewed interest in physiological experimentation using Porifera. To-day the emphasis is on war-winning, but ultimately the lowly animals may again receive attention.

Unfortunately the names of Porifera require some notice. It is common practice for text-book writers to copy the mistakes made in earlier text-books until accuracy becomes an irritating intrusion.

Consider the commonest commercial or bath sponge genus. Linné in his "Systema Naturae" named it *Spongia,* and for exactly a century it was always called that. In 1859 a person named H. G. Bronn decided he didn't like Linné's name, and arbitrarily announced that it should be changed to *Euspongia.* Because Bronn was a professor in Heidelberg University, he was meekly followed in his utterly unwarranted act, and to this day most texts of zoology call it by his presumptuous name. Shall we pick out some American University and say that its professors may change scientific names at their whims? The correct name is still *Spongia.*

The second most common commercial or bath sponge has had a miserable time with names. F. E. Schulze in 1879 described it in general, and named it *Hippospongia,* and so it has been known. Yet it seems that Professor Schulze failed to set up a type specimen, and therefore in 1934 Maurice Burton designated a specimen as type of *Hippospongia.* But alas, Dr. Burton's specimen is of the other genus, that is to say, a *Spongia.* Now we begin to go around in dizzy circles. If Burton has the right to select the type specimen (and this is probable) then *Hippospongia* falls in synonymy.

A partial solution occurred to me. In 1936 I de-

scribed and named this extremely important genus by the name of *Hippiospongia,* adding only the one letter "i." Also there is a good type specimen in the National Museum to bind it. Now if Burton's action is valid, my name stands, and if not, Schulze's name is still good, and in either case the two names are so nearly identical that there should be no doubt as to what sponge sort is being discussed, with or without the extra letter in its name.

The fresh-water sponges are widely available to zoologists, and are therefore much noticed. Their first genus is *Spongilla* of Lamarck, 1815; well and good. However, a year earlier in 1814 a certain L. Oken had described a fresh-water sponge and given it the genus name *Tupha.* Then in 1816 Lamouroux set up another name *Ephydatia,* about which the one certain thing is that it is a synonym of *Tupha.* Lamouroux wasn't describing a specimen at all. He just made a name, and specifically referred to the identical specimen to which Oken had referred.

Spongilla is the main fresh-water sponge, but there is a second sort, nearly as common and important. Carter in 1881 described it well and named it *Meyenia.* A few years later some one named Vejdovsky got the notion, with no evidence whatsoever, that this *Meyenia* should be called *Ephydatia,* and to-day he is widely copied. If it were the same genus as *Ephydatia* (which is anybody's guess) it would certainly have to be *Tupha.* Yet in complete default of specimens, and with the utterly unrecognizable descriptions of the ancient authors, there is absolutely no justification for digging up the ancient names at all. The genus is *Meyenia;* first, last and always.

My article in SCIENCE for February 19, 1937, pointed out that the common Calcisponge that is used as standard class material in America had been carelessly mis-identified, and is really *Scypha,* not *Grantia.* There is a common European sponge correctly named *Grantia,* but not at all the same as the one we use so much in this country. Now recent text-books of zoology are calling this genus still another name, *Sycon.* It is a pity to have to dig up the history of this set of names, but apparently necessary. The name *Scypha* was given to this sponge in 1821 by J. E. Gray, but like so many biologists of that era, he thought that all sponges were vegetable, therefore he stated that this was a genus of plants. Five years later, in 1826, Mr. A. Risso decided that Gray was wrong, and that since the sponge had been named as a plant it was fair game for a new name as an animal. So he proposed to call it *Sycon.* Yet it has long been regarded that the sort of mistake Gray made does not invalidate the name he gave. We may not like the name *Scypha,* but unless official international action is taken, it is correct.

The term "syeon" is well established as the name of a certain type of sponge architecture. It is therefore here suggested that it would not be wise to have the earlier name set aside in its favor.

It has long been recognized that there are three classes of sponges. The prior names for these are

(1) Calcispongiae—Blainville 1830 (or Schmidt 1862).
(2) Vitrea—Wyville Thomson 1868.
(3) Demospongiae—Sollas, 1875.

Priority does not need to be followed in regard to class names, although wanton shifting about is deplored. In 1886 Vosmaer proposed changing the second class from Vitrea to Hyalospongiae, which gives us three classes of the sponge phylum, all ending in the syllables "-spongiae." This harmony is very helpful to students, and it is therefore probable that Vosmaer's change does deserve to be followed.

Since the turn of the century there has been a growing tendency to alter the first name to Calcarea and to alter the second name further from Vitrea to Hexactinellida. It is not clear at all what advantage (if any) is served by these changes. There is, and has been for a very long time, in the Hyalospongiae a family named Hexactinellidae. In my publications it has therefore been suggested that it would be most helpful to adhere to the long-established class names: Calcispongiae, Hyalospongiae and Demospongiae.

M. W. DE LAUBENFELS

NOTES ON RUSSIAN AND OTHER EUROPEAN HERBARIA

FROM a communication recently received from Leningrad it is reported that the very large and important herbarium and library at the Komarov Institute of Botany (Principal Botanical Garden) suffered no damage during the siege of Leningrad although many bombs fell in the grounds. Most of the living greenhouse collections were lost because of breakage of glass by bombs. Likewise the Siberian part of the important Turczaninow herbarium at Karkov was removed to Leningrad, and is thus safe. The general Karkov herbarium, however, the Ukranian Academy of Science at Kiev and the Nikita Botanical Garden near Yalta in the Crimea were confiscated by the German invaders and moved to Germany.

The one important herbarium and botanical library in France that we know to have been utterly destroyed is that of the University of Caen, for the university buildings were wrecked during the invasion of Normandy. However, it is reported from Paris that nothing was disturbed in the great historical collections at the Muséum d'histoire naturelle.

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JAMAICA PLAIN, MASS.,
MARCH 29, 1945

SCIENTIFIC BOOKS

TROPICAL MEDICINE

Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases. By RICHARD P. STRONG, M.D., Sc.D., D.S.M., C.B. Seventh ed. 2 vols. pp. xiv + 1747 + xl. 397 figs., 4 color plates. The Blakiston Company. 1944. $21.00.

THIS American text-book has been a necessary part of the library of every student of tropical medicine since Admiral E. R. Stitt brought it into being in 1914. When Colonel Strong undertook the task of preparing the sixth edition, he was able to add a wealth of personal knowledge and an experience of nearly fifty years in this field. Thus, in January, 1942, at a critical time in the development of American military operations in tropical areas, there became available a relatively exhaustive reference book on the diseases of warm climates. The sixth edition went through four printings and now the newer knowledge in the field is incorporated with the standard subject-matter.

It is neither possible nor necessary to elaborate on the diseases included in this extensive work. It is sufficient to state that it includes an able presentation of each of the many diseases produced by the protozoa, bacteria, filtrable viruses, rickettsias, fungi and poisonous plants, helminths, arthropods and other parasitic or poisonous animals. Nutritional diseases which constitute so important a part of the social and disease economy of native populations in tropical and Oriental countries are rather briefly but clearly considered. In addition, one section is devoted to "diseases not satisfactorily grouped in other sections," a chapter on the methodology of medical practice in the tropics, including the cosmopolitan diseases present in tropical countries. The appendix includes sections on clinical diagnosis, laboratory diagnosis, one each on group and personal hygiene and one on disinfectants and disinfestants.

There is no particular adverse criticism which is merited by this two-volume contribution, although a rearrangement of the section groups might have been advisable. For example, there might logically be an introductory section on climates in warm countries, their direct and indirect effects on human beings and methods of determining the existence and amount of illness and death among native peoples in the tropics. This would lead up to infectious diseases, which are so adequately presented, followed by deficiency diseases and a more adequate consideration of cosmopolitan diseases and their epidemiology in warm climates. The material in the appendix can not readily be integrated into the body of the text and constitutes valuable supplementary reference information. The size of the publication probably precludes more reference citations, but a reader not living in a center with extensive library facilities would be materially aided if more of the sources cited in the text were included in the "references" at the end of each chapter.

The two volumes are clearly printed on good paper, the format is pleasing, the illustrations are for the most part very good, and the binding is moderately strong and durable. Numerous typographic errors and minute inconsistencies which escaped the proofreader in the sixth edition have been eliminated. Both Colonel Strong and the publishers are to be thanked for this modern, distinguished contribution to American medicine.

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FOOD AND FOOD PRODUCTS

The Chemistry and Technology of Food and Food Products. Prepared by a group of specialists under the editorship of MORRIS B. JACOBS, Ph.D., senior chemist, Department of Health, City of New York. Set of 2 volumes. Volume I—xviii, 952 pages, including 79 illustrations and 218 tables—Volume II—xx, 890 pages, including 166 illustrations and 84 tables. New York: Interscience Publishers, Inc. 1944. $10.50 per single volume; $19.00 for the set of 2 volumes.

THE volumes under review constitute a broad treatment of the chemistry and technology of food and food products. The field is covered by 48 chapters well printed on good paper. Forty-one collaborators have prepared a unified authoritative work by approaching the problem from the point of view that an expert in any selected subject is best qualified to write about. Thus, to cover the various phases of the subject-matter, food technologists, chemists, biochemists, bacteriologists, sanitary engineers, public health officers, food inspectors and entomologists are represented among the contributors.

The two volumes are divided into a total of six parts. The first, on fundamentals, deals with the aspects of food chemistry which are common to all foods. The second part concerns the descriptive aspects of particular food groups, and includes some account of the history, statistics, definitions, standards, composition and chemistry of these food groups. In part three, unit operations and processes applicable to most foods are described. Part four deals with the maintenance of sanitary and quality control of foods and food products. In part five, the principal methods of preserving foods are delineated. Part six is concerned with production methods for the principal foods.

The editor and the collaborators are to be congratulated upon the excellent and comprehensive job which they have done. These encyclopedic volumes will be greatly appreciated as books of reference by

all those interested in the production, chemistry, sanitation and control of foods. They will serve also as interesting and informative reading sources by those who are not food specialists.

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FATS AND OILS

Fats and Oils (An Outline of Their Chemistry & Technology). By H. G. KIRSCHENBAUER. 154 pp. New York: Reinhold Publishing Corporation. 1944. $2.75.

THIS book represents one of the first attempts to present briefly both the chemistry and technology of the vegetable fats and oils. Of the 140 pages of written material, about one third is given to an outline of the structure of fats and fatty acids and to analytical methods; about 35 pages are used for descriptive notes and general characteristics of the more important fats; and the remainder of the book deals with technological aspects such as methods of production, purification, hydrogenation, hydrolysis, distillation, spoilage, detergents and lubricants. Although such a condensed treatment offers very little new material and information not previously available in the accepted references and monographs, it does provide a satisfactory introduction to the extensive field of fats and oils. Its use will be of very limited value to those engaged in either chemical or technological work with fats and oils.

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SPECIAL ARTICLES

NEW STRAINS OF PENICILLIUM NOTATUM INDUCED BY BOMBARDMENT WITH NEUTRONS[1, 2]

MODERATELY heavy spore suspensions in normal saline in test-tubes of a stable variant isolated from a strain of *Penicillium notatum*[3] were bombarded with neutrons from the 42-inch cyclotron of the physics department. Tubes were removed after 1, 2, 3.5, 4.5, 5, 6 and 7 hours on the first day when the beryllium target (other targets were also used subsequently) was being bombarded by 10 mev deuterons. On succeeding days, after increasing the total time of operation of the machine, additional tubes were removed from time to time.

RESULTS

Approximately 100 new strains or mutations have been isolated from cultures of the bombarded spore suspensions. This was done by comparing colony characteristics such as color, size and texture with those of controls which received no neutron bombardment.

Some of the new strains isolated have been cultured simultaneously in large batches on the surface of medium in half-gallon milk bottles by the well-known methods. Under identical conditions such as medium, time and temperature there have been found marked differences with respect to rate of sporulation; yields of antibiotic activity when compared by the cup assay method; and color and texture of the mats among some of the strains isolated.

As a typical example the results of quadruplicate cup assays of composites of several gallons each of surface-culture metabolism liquor from two different strains isolated after neutron bombardment are given in Table 1.

TABLE 1

Green Mat	Buff Mat
86	153
90	128
60	143
86	143
Aver. 80.5	142 Oxford units

Although the eventual yield of antibiotic activity by the buff mat strain was superior to the green mat strain by 76 per cent., the rate of sporulation of the green mat was much faster than in the case of the buff mat strain during the early stages of culture.

Preliminary experiments indicate that the antibiotic activity produced by many of the new strains does not differ qualitatively from that produced by the parent strain with respect to inhibition of growth of various bacterial species. Work is being continued to rule in or out the possibility that new antibiotics are being produced by the mold as the result of neutron bombardment.

The number of new strains which appears is in some measure proportional to the amount of bombardment. Thus only a few new strains were found in cultures of spores removed during the first day of bombardment, whereas new strains were very numerous in cultures of spores bombarded by neutrons each time the cyclotron was in operation (approximately 5 days each week) over a period of 4 months.

At present we are exposing suspensions of spores

[1] This study is a contribution from the Departments of Chemistry and Bacteriology of the Ohio State University under fellowships sponsored by the Wm. S. Merrell Company and administered by the Ohio State University Research Foundation.

[2] Experimental method.

[3] Obtained from the Northern Regional Research Laboratory.

of *Actinomyces lavendulae*[4] and *Actinomyces griseus*[4] to neutron bombardment to see if new strains will similarly result which can advantageously be used to produce more streptothricin and streptomycin respectively and in less time or both or whether new strains will appear which will yield new and more desirable antibiotics. Preliminary results are promising in that new strains are appearing, but less frequently than in the case of *P. notatum*.

We propose similarly to bombard other micro-organisms with neutrons, especially bacteria, molds and yeasts of industrial and medical importance to see if new strains can be isolated which will find advantageous application in industrial and medical microbiologic processes.

A more detailed presentation of the substance of this communication will be submitted for publication elsewhere.

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THE DIFFERENTIAL DIAGNOSIS OF BOVINE BRUCELLOSIS FROM THE BACTERICIDAL ACTION OF BLOOD PLASMA

IN herds of cattle in which several animals are infected with *Brucella abortus*, it has been a common observation that many develop only low serum agglutination titers. According to the standards in vogue to-day, such animals are placed in a border-line category as regards active infection. A long time-interval must intervene and several tests be made before the actual status of such animals can be determined. Very often such a practice is not economical and, too, such animals are considered unsafe when valuable dairy cattle are involved.

It has been observed that most of the animals in the suspicious category, in herds in which infected ones have been present for many years, will in time become negative to the agglutination test. In the herd in which infection is of recent date, it has not been possible by procedures now in use to predict the future of those that show low agglutination titers.

In a comprehensive *in vitro* study of the bactericidal and growth-inhibiting action of bovine blood plasma for *Br. abortus*, sufficient differences were found in the action of plasma from infected and non-infected cows to differentiate one from the other regardless of the agglutination titer. The latter group contained normal animals and animals showing different agglutination titers from a previous natural exposure, or following the injection of a killed *Br. sius* vaccine.

[4] Obtained from Dr. S. A. Waksman, Rutgers University.

[1] Part of a cooperative project between Michigan State College and the Bureau of Animal Industry of the U. S. Department of Agriculture.

PROCEDURE EMPLOYED AND RESULTS

Blood was collected from the jugular vein by means of a sterile needle into sterile bottles containing 0.1 ml of saturated sodium citrate for each 10 ml of blood. Plasma was separated from the cells by centrifugation and tested immediately. If necessary, the sample may be stored at 4° C. for as long as 10 days without impairing its bactericidal action.

A single, smooth strain of *Br. abortus* was used in all experiments. The differential value of the growth inhibition test depends upon the employment of a smooth culture. If a dissociated culture is used, plasma from both infected and non-infected animals will inhibit its growth to an equal degree.

The organisms were grown on beef liver agar slants for 24 hours at 37° C., then removed by means of a wire-loop and suspended in distilled water containing 0.05 per cent. Tryptose peptone and 0.5 per cent. NaCl. The bacterial suspension was then diluted to a scale reading of 28 on the Libby Photronreflectometer. The standard suspension contained approximately 1.5×10^9 live *Brucella* organisms per ml. This was diluted 1:1,000 in the same diluting liquid for final use.

The outline of the method which has proved to be the most practical and the least involved, and at the same time reveals more distinct differences between the action of plasma of normal, immune and infected animals follows: Each plasma sample was diluted two-fold in tubes of Tryptose broth from a 1:10 dilution to 1:1,280; the final volume in each tube was 5 ml plus 0.1 ml of diluting fluid containing 1.5×10^5 live organisms. The tubes were incubated for 48 hours at 37° C. The results were recorded at the 24th and 48th hour. The action of the plasma was measured by the absence or degree of growth (turbidity) in the tubes as compared with that in a control tube inoculated at the same time. The organisms multiplied sufficiently during the first 24 hours to produce considerable turbidity in the control tube of medium. The medium remained clear in those tubes in which growth was completely inhibited.

In Table 1 are set forth results representative of those that have been obtained on plasma samples from a large number of cattle of different statuses toward *Brucella* infection. Not in a single instance has a plasma sample from infected animals inhibited the growth of *Br. abortus* in any of the dilutions employed routinely. That this is not an exhibition of "zone-inhibition phenomenon" has been repeatedly demonstrated by the failure to obtain inhibition of growth in further two-fold dilutions of the plasma up to 1:200 million even after adding bovine or guinea pig plasma to each dilution to provide complement.

The *Brucella* organisms always grow in aggregates

TABLE 1

THE GROWTH-INHIBITING ACTION OF BLOOD PLASMA FROM DIFFERENT ANIMALS AGAINST *Br. abortus*

Source	Plasma sample: Agglutination titer	Hours of incubation	Dilution—growth inhibition: 1:10	1:20	1:40	1:80	1:160	1:320	1:640	Control
Normal calf age, 2 weeks	–	24	–	–	–	–	–	–	–	2+
		48	–	–	–	–	–	4+	4+	4+
Normal heifer age, 15 months	–	24	–	–	1+	2+	2+	2+	2+	2+
		48	1+	4+	4+	4+	4+	4+	4+	4+
Heifer, recovered from infection	1:25	24	–	–	–	–	–	1+	2+	2+
		48	–	–	–	–	–	4+	4+	4+
Heifer, 1 month after vaccine treatment	1:1,280	24(a)	–	–	–	–	–	1+	2+	2+
		48(a)	–	–	–	–	–	4+	4+	4+
Infected cow	1:1,280	24(a)	2+	2+	2+	2+	2+	2+	2+	2+
		48(a)	4+	4+	4+	4+	4+	4+	4+	4+

– = Tubes remain clear, no growth.
+ = Degree of growth.
(a) = Growth in aggregates.
Number of organisms added to each tube per ml = 1.5×10^5.

in the plasma dilutions of infected animals up to the corresponding agglutination titer of the sample. This also occurs beyond the zone of inhibition in samples from animals that have been injected with vaccine. When the aggregates of organisms, in the latter, are transferred to agar plates, most of the colonies grow up as dissociants. Transfers of growth in all dilutions of plasma from infected cows remain smooth.

Plasma samples from most young calves inhibit growth in all dilutions up to 1:160. Occasionally calves are encountered whose plasma shows little inhibition of growth in any dilution. The presence of bactericidal substances and degree of action of plasma in the new-born calf has been correlated with the ingestion of colostrum. Before the ingestion of colostrum the blood possesses no growth-inhibiting property for *Brucella*.

Plasma samples from heifers and adult cows after exposure to and recovery from natural infection inhibit the growth of *Br. abortus* in a higher dilution than those from normal ones. This also occurs in plasma from cows injected with a new type of killed *Brucella* vaccine. It is possible that these observed differences in the growth-inhibiting power of plasma can be made use of in determining the resistance of an animal to *Brucella* infection as well as in detecting those that are infected, as the studies conducted by Irwin, Beach and Bell,[2] and Irwin and Ferguson[3] have suggested.

Thus far, the growth inhibition test has proved to be a highly accurate means of identifying both young and adult cows that are infected with *Br. abortus*, and whose agglutination titers range from 1:25 to 1:5,000.

The test can easily be developed into a routine laboratory procedure and by its application bring about the retention of many cattle that might otherwise be disposed of because of the possibility of infecting other cattle.

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[2] M. R. Irwin, B. A. Beach and F. N. Bell, *Jour. Inf. Dis.*, 58: 15–22, 1936.
[3] M. R. Irwin and L. C. Ferguson, *Proc. Soc. Exp. Biol. and Med.*, 38: 451–452, 1938.

DETOXICATION OF ARSENIC TRIOXIDE BY ORAL ADMINISTRATION OF FLUORIDES

PHYSIOLOGICAL antagonism between arsenic and various chemical compounds has been demonstrated by a number of investigators. The work of Rosenthal and Voegtlin[1] indicates that reduced glutathione is an effective antidote against arsenoxide and arsenious acid. Moxon and his associates[2] have markedly reduced the toxicity of selenium by treatment with sodium arsenite. Sandground[3] has found p-aminobenzoic acid to be highly effective as a detoxicant for high lethal doses of carbarsone and certain other phenyl arsonates in rats. Ascorbic and other organic acids are known to exert a protective action against neoarsphenamine.[4] Arsenic has been suggested as a causative factor in goiter and has been shown to be antagonistic to iodine.[5,6]

The experiments to be described in this paper are an attempt to demonstrate *in vivo* antagonism between two elements, arsenic and fluorine, using arsenic in one of its most virulent forms, the inorganic trivalent arsenious acid.

The arsenic trioxide (As_2O_3) was administered

[1] S. M. Rosenthal and C. Voegtlin, *Jour. Pharmacol. and Exp. Therap.*, 39: 347, 1930.
[2] A. L. Moxon and M. Rhian, *Physiol. Rev.*, 23: 305, 1943.
[3] J. H. Sandground, SCIENCE, 97: 73, 1943.
[4] E. W. McChesney, O. W. Barlow and G. H. Klinck, Jr., *Jour. Pharmacol. and Exp. Therap.*, 80: 81, 1944.
[5] M. Scott, Third Int. Goiter Conf., *Trans.*, 34, 1938.
[6] G. R. Sharpless and M. Metzger, *Jour. Nutrition*, 21: 341, 1941.

orally by mixing with powdered sugar and allowing the rats to eat it voluntarily. In almost all cases the complete amount was consumed within two days. The rats which received fluoride were supplied with potassium fluoride (KF) in the drinking water. A 24-hour pretreatment was given to all rats during which they were starved, and the experimental group supplied with the fluoride-containing drinking water. The arsenic-sugar mixture was then placed in individual cages and the rats were observed for the succeeding 10 days. No supplementary food was given until all rats had consumed their sugar supply. At the end of the fifth day the experimental rats were returned to ordinary tap water.

Nineteen rats were fed 30 mg As_2O_3, and of these only 3, or 16 per cent., were alive on the fifth day of the experiment. These same 3 rats lived through the remainder of the 10-day period.

Sixteen rats were fed 30 mg As_2O_3 and were supplied with drinking water containing 15 mg KF per 30 cc of water. All these animals were alive on the fifth day, and 14, or 88 per cent., were alive at the termination of the experiment.

Twelve rats were fed 30 mg As_2O_3 and were supplied with drinking water containing 30 mg KF per 30 cc of water. Of this group 2 rats died during the first 5 days, while the remaining 10, or 83 per cent., survived the experiment.

These results have been analyzed statistically, using the appropriate chi-square test at the .01 level of significance. The results of the two fluoride-treated groups do not differ significantly, but each of them is significantly different from the control group.

The mechanism of action of arsenic on protoplasm as suggested by Voegtlin[7] consists of an interference with normal functioning of glutathione in the oxidation-reduction processes in living tissues. The injection of a toxic dose of arsenic leads to cellular asphyxia, possibly due to a chemical reaction between reduced glutathione and arsenic to form a compound incapable of normal respiratory activity. Rosenthal and Voegtlin[1] showed that glutathione administration can protect rats against a lethal dose of arsenic, probably substituting for the amount tied up by arsenic. They found that feeding of fairly large amounts of glutamic acid and cystin, precursors of glutathione, will give considerable protection against a subsequent minimal lethal dose of arsenic.

It is suggested by Hogan and Eagle[8] that the systemic toxicity of arsenicals is primarily determined by the varying degree to which they are bound by, and thus block, essential functional groups in vital organs. These assertions are in accord with Ehrlich's thesis that chemotherapeutic agents in general can exert their therapeutic effect only if bound by the parasite, and that their toxic action is due to a similar combination with vital tissues of the host.

The chemical compounds which have been described as detoxicants for arsenicals presumably perform their function by a variety of methods. Organic acids, such as ascorbic and lactic acid, appear to exert their effect as detoxicants of neoarsphenamine by preventing its *in vivo* oxidation to highly toxic inorganic oxides.[4] It has been suggested that because p-aminobenzoic acid bears a similar structural relationship to arsanilic acid as it does to sulfanilamide an explanation of the mechanism of detoxication may be found which parallels the enzyme blockade theory.

The presence of fluoride in the tissues of an arsenic-fed animal must in some way prevent the binding of an essential substrate, such as the physiologically essential sulfhydryl groups. There may be formation of a compound of arsenic and fluorine which has less affinity for body tissues, or is less readily absorbed, so that the arsenic is bound and excreted before it can attack a vital system. The concept of reduced absorption is strengthened by the conclusions of Smith and Shaner,[9] who prevented death of guinea pigs fed a double lethal dose of fluoride by buffering with calcium carbonate.

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DEPRESSION OF LYMPHOCYTE CONTENT OF THORACIC DUCT LYMPH BY ADRENOCORTICOTROPHIC HORMONE

EXPERIMENTAL evidence available at the present time has demonstrated a reciprocal relationship between the adrenal cortical functional state and the weight of lymph and thymic tissue present in the body. Thus adrenalectomy has been shown to result in increase in weight of thymus and lymph nodes as compared with the normal control rat.[2] On the other hand, stimulation of the adrenal cortex by administration of adrenocorticotrophic hormone (ACTH) or the administration of adrenal cortical extracts has produced a rapid decrease in the weight of thymus and lymph nodes in the mouse and rat.[3, 4]

[7] C. Voegtlin, *Physiol. Rev.*, 5: 63, 1925.
[8] R. B. Hogan and H. Eagle, *Jour. Pharmacol. and Exp. Therap.*, 80: 93, 1944.
[9] R. R. Smith and E. O. Shaner, *Jour. Am. Dent. Ass.*, 31: 1483, 1944.

[1] Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City.

[2] W. O. Reinhardt and R. O. Holmes, *Proc. Soc. Exp. Biol. and Med.*, 45: 267, 1940.
[3] T. F. Dougherty and A. White, *Proc. Soc. Exp. Biol. and Med.*, 53: 132, 1943.
[4] M. E. Simpson, Choh Hao Li, W. O. Reinhardt and Herbert M. Evans, *Proc. Soc. Exp. Biol. and Med.*, 54: 135, 1943.

It might be expected that such pronounced changes in the wide-spread lymphatic system would result in definite and marked changes in the blood leucocyte picture, particularly as regards the lymphocytic elements. Such a change has been demonstrated experimentally in the mouse, rat and dog.[5, 6] Injection of ACTH or adrenal cortical extracts into these forms has resulted in the production of an absolute lymphopenia.

The mechanism of production of this lymphopenia has been of sufficient interest to stimulate the following experimental approach. Single doses of pure ACTH (1–6 mg dose levels) were injected (by subcutaneous and intraperitoneal routes) into normal and adrenalectomized adult female rats of the Long-Evans strain. The hormone (in amounts of 6 mg per cc) was dissolved in a phosphate buffer solution of pH 7.4. To measure the flow of thoracic duct lymphocytes into the blood stream, the common lymph sac, into which empties the thoracic duct in the rat, was cannulated[7] intermittently, before and after injection of the various hormone preparations. The animals were anesthetized by intraperitoneal injection of a 1 per cent. solution of sodium pentobarbital. Leucocyte counts were carried out on thoracic duct lymph specimens at intervals of 30 to 60 minutes for a number of hours preceding and following administration of ACTH.[8]

The results of the experiments obtained to date justify the following conclusions. Administration of ACTH by either the subcutaneous or intraperitoneal route produced a rapid decrease in the number of lymphocytes in the thoracic duct lymph. This reduction occurred within 15 to 30 minutes, was usually more than a 50 per cent. reduction in the pre-existing cell count, and persisted in most rats for the duration of the experiment (4 to 10 hours). Administration of ACTH to the adrenalectomized animal did not produce a comparable effect. Subcutaneous injection of pure growth[9] and lactogenic[10] in similar dose levels failed to imitate the action of ACTH. The buffer solution and the anesthetic agent employed produced no demonstrable effect on the lymphocyte level of the thoracic duct lymph. There were no changes in the rate of flow of the lymph which could account for changes in the lymphocyte level in the thoracic duct.

It is apparent from the results of these experiments that stimulation of the adrenal cortex by the administration of ACTH produces a rapid and persisting fall in the number of lymphocytes entering the blood stream, probably as the result of decreased outpouring of these cells from the thymus and lymph nodes. This change can, it is felt, account in a large measure for the lymphopenia of absolute proportions in the blood stream of the rat following injection of ACTH. Further experiments must clarify the fate of the lymphocyte circulating in the blood stream. A direct experiment to demonstrate the effect of the adrenal cortex on lymphocyte production would consist in the collection of lymph from the isolated lymph node with intact artery and vein in the living animal before and after administration of ACTH or adrenal cortical extract. It will be of further intense interest to determine which of the substances present in the adrenal cortex are responsible for this rather dramatic effect on the lymphocyte level of thoracic duct lymph. The technique of measuring the fall in lymphocytes in thoracic duct lymph may further prove to be a sensitive indicator for the assay of ACTH or adrenal cortical substances.

It may be generalized that the lymphocyte level of the circulating blood is under direct adrenal cortical control. Such a generalization should divert attention to the role of the anterior pituitary and adrenal cortex in various disease states accompanied by hitherto inexplicable relative and absolute lymphopenias or lymphocytosis. To the knowledge of the writers, this is the first direct experimental demonstration using pure anterior pituitary hormones of the control of entry of a white blood cell into the circulating blood stream.

This is a preliminary communication.

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[5] T. F. Dougherty and A. White, SCIENCE, 98: 367, 1943.
[6] W. O. Reinhardt, H. Aron and Choh Hao Li, *Proc. Soc. Exp. Biol. and Med.*, 57: 19, 1944.
[7] W. O. Reinhardt, "Rate of Flow and Cell Count of Rat Thoracic Duct Lymph." To be published.
[8] Choh Hao Li, M. E. Simpson and H. M. Evans, SCIENCE, 96: 450, 1942.
[9] Choh Hao Li and H. M. Evans, SCIENCE, 99: 183, 1944.
[10] Choh Hao Li, M. E. Simpson and H. M. Evans, *Jour. Biol. Chem.*, 146: 627, 1942.

SUSCEPTIBILITY OF HAMSTERS TO CLOSTRIDIUM CHAUVEI

IN experimental work with *Cl. chauvei* where clinical infection is necessary, guinea pigs are generally employed. In comparison to cattle where the basic infection occurs naturally, an immensely larger dose of the infecting agent, on a basis of body weight, is necessary. Berg[1] notes that guinea pigs are comparatively insusceptible to blackleg and quotes Nitta's statement that it required as much culture to kill guinea pigs as calves. He also states that Graub and Zschokke found it took twice as much to kill guinea pigs as calves and sheep.

Considerable work with one strain of *Cl. chauvei* disclosed that 0.3 cc of a 48-hour Hibler culture injected

[1] W. N. Berg., *Jour. Am. Vet. Med. Asn.*, 607–622, 1922.

intramuscularly would uniformly kill adult guinea pigs in 24 to 48 hours. 0.25 cc either failed to kill or was fatal only after 4 or 5 days. Differences in body weight had no apparent effect on these amounts.

Inasmuch as hamsters are finding increasing use as laboratory animals because of their greater susceptibility to many infections, it was decided to compare their susceptibility to that of guinea pigs. Recently weaned pigs were employed so their weight would be closer to that of the young adult hamsters, although the possibility of some passive immunity from the sow would have to be considered. However, within two weeks of weaning their susceptibility has been found identical to that of adults.

TABLE 1

Amount*	Weight	cc Kg	Inoculation route	Results
		GUINEA PIGS		
0.3 cc	200.5 gms	1.49	Axilla	Dead in 24 hrs.
0.25 "	195.5 "	1.27	"	Dead in 48 hrs.
0.2 "	143.5 "	1.39	"	Survived
0.15 "	219.5 "	0.68	"	"
0.5 "	209.5 "	2.38	Orally	"
		HAMSTERS		
0.2 cc	71. gms	2.81	Thigh	Dead in 48 hrs.
0.1 "	55. "	1.81	"	" " " "
0.05 "	49.5 "	1.01	"	" " " " "
.01 "	51.5 "	0.19	"	Survived
.001 "	61.5 "	0.01	"	"
0.3 "	50.5 "	5.94	Orally	"

* 48 hr. Hibler culture *Cl. chauvei*.

Table 1 again indicates that the M. L. D. for guinea pigs can not be stated in terms of body weight but exists as an animal unit. Hamsters were found to be more susceptible to *Cl. chauvei* infection than guinea pigs, succumbing to one fifth the lethal dose required for guinea pigs on an animal unit basis.

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THE GERMICIDAL EFFICIENCY OF EMULSEPT AND OF CHLORINE IN WASHING DIRTY EGGS[1]

RESULTS of previous experiments performed in this laboratory on the germicidal efficiency of one of the new types of quaternary ammonium cationic compounds, namely emulsept, have been reported by us.[2] At the same time Zagaevsky and Lutikova published data on the germicidal efficiency of chlorine in disinfecting egg shells.[3] This present paper concerns the comparative values of chlorine and emulsept as germicides in washing dirty eggs.

A. K. Epstein *et al.* have indicated that emulsept, which is the lauric acid ester of colamino formyl methyl pyridinium chloride, has the low toxicity index of 0.6[4] and that the compound, in addition to being a good detergent, is colorless, odorless and non-irritating to the skin.[5]

EXPERIMENTAL METHODS

Eggs used in our experiments were candled out at the receiving station as medium to heavy dirty eggs, the shell surfaces of which were covered with soil, chicken excreta, feathers, straw and egg meat.

Two types of controls were used for later washings with emulsept and chlorine. First, 72 dozen heavy dirty eggs were washed in 6 liters of tap water in 6 groups of 12 dozen each. After washing each 12 dozen eggs the number of organisms present in the wash water was determined by plate counts. Second, from each group, 6 eggs (2 from the top, middle and bottom of the pail) were transferred to 600 ml of sterile water. The jars were rotated rapidly in order to swirl the water over the egg-shell surfaces. This water was then plated, incubated at 30° C. for 3 days and the number of colonies of microorganisms counted. The counts from these plates gave an indication of the number of organisms carried over on each egg shell from the dirty wash water into the rinse water. Seven trials of each series were made for both methods and the results were averaged to be used as controls.

When the eggs were washed with the disinfectants the same procedures were followed with the exception

TABLE 1

COMPARISON OF GERMICIDAL EFFICIENCY OF EMULSEPT AND CHLORINE IN WASHING DIRTY EGGS

Germicide used	Successive groups of 12 dozen eggs each	Percentage of microorganisms not killed by germicide in relation to the number of microorganisms present when no disinfectant was used		Average concentration of germicide remaining
		In rinse water	In wash water	
Emulsept 0.04 per cent.	1	0.26	0.24	*
	2	1.12	0.90	
	3	3.52	3.13	
	4	3.73	6.97	
	5	8.00	33.00	
	6	17.58	36.65	
Chlorine 100 ppm	1	20.28	12.70	4 ppm
	2	13.80	10.54	0.4
	3	40.80	52.45	0.0

* When these experiments were conducted no method had been devised to measure the amount of emulsept remaining in the wash water.

[1] Preliminary report.

[2] V. Penniston and L. R. Hedrick, *U. S. Egg and Poultry Mag.*, 50: 26, 1944.

[3] J. S. Zagaevsky and P. O. Lutikova, *U. S. Egg and Poultry Mag.*, 50: 17, 1944.

[4] A. K. Epstein, B. R. Harris and M. Katzman, *Proc. Soc. Exp. Biol. and Med.*, 53: 238, 1943.

[5] A. K. Epstein, B. R. Harris, M. Katzman and S. Epstein, *Oil and Soap*, 20: 171, 1943.

that (1), 8 trials each with emulsept and chlorine were made rather than 7 as in the controls, and (2), with 100 ppm active chlorine it had been found by preliminary experiments to be necessary to use only 3 groups of the 12 dozen eggs rather than 6 groups. The percentages of microorganisms (in relation to the number in the controls) remaining in the rinse water and in the wash water after using the two different disinfectants are given in Table 1.

SUMMARY

The percentages of microorganisms killed with emulsept in these experiments for the same series of eggs are many times greater than the percentages of those killed when 100 ppm active chlorine was used. Experiments are in progress on other phases of this problem.

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NEW MATERIALS FOR DELAYING FRUIT ABSCISSION OF APPLES

IN experiments performed in 1939,[1] growth-regulating chemicals as sprays proved highly successful in delaying the harvest drop of apples. Of a number of compounds tested, naphthaleneacetic acid, naphthaleneacetamide and the metallic salts of naphthaleneacetic acid have proved to be the most outstanding in effectiveness. Extensive use of these sprays by orchardists has been exceedingly helpful in reducing losses from harvest drop of apples and pears. However, the limited period of effectiveness of the sprays (usually from 10 days to 3 weeks), with consequent necessity of rather exact timing of the applications, has been the major difficulty in the successful use of these sprays. To increase both the duration and the intensity of the effect of such a spray application, the action of some compounds not heretofore tested was determined in the fall of 1944.

EXPERIMENTAL RESULTS

The data, expressed as accumulated per cent. drop at frequent intervals following the spray applications on Winesap apples are shown in Table 1. Until 15 days after treatment both naphthaleneacetic acid treatments, with and without the addition of Carbowax (a polyethylene glycol), were highly effective in preventing fruit drop. While 4-chlorophenoxyacetic acid and 2,4,5 trichlorophenoxyacetic acid reduced drop significantly during this period, these treatments were not so effective as the 2,4 dichlorophenoxyacetic acid spray. Beginning 15 days following application, the rate of fruit drop with the naphthaleneacetic acid treatment showed a marked increase, and by 17 days the effect of the treatment had been almost or entirely dissipated, since the fruit drop from that date until the completion of the experiment was at approximately the same rate as for the untreated trees. The addition of Carbowax to naphthaleneacetic acid, however, considerably extended both the intensity and duration of effect. The rate of fruit drop for trees receiving this treatment did not approximate the rate of drop for the untreated trees until 23 days after application. The 2,4 dichlorophenoxyacetic acid treatment, while somewhat less effective than the naphthaleneacetic acid treatments for a considerable period following the spray applications, was effective over a period nearly double that of naphthaleneacetic acid alone, and several days longer than for naphthaleneacetic acid plus Carbowax. On October 30, when the fruit was harvested, most of the apples on the trees receiving 2,4 dichlorophenoxyacetic acid treatment were firmly attached to the spurs, and while pulling them off was not difficult, there was no suggestion that the fruit was any "looser" than it had been several weeks earlier. In contrast to this condition, the fruit on the trees of the next most effective treatment (naphthaleneacetic acid plus Carbowax) would drop from the tree at the slightest touch.

TABLE 1

EFFECT OF GROWTH-REGULATING CHEMICALS ON HARVEST DROP OF WINESAP APPLES

Spray treatment	Average accumulated per cent. drop Days following spray application					
	7	11	15	19	23	28
.001 per cent. naphthaleneacetic acid	0.6	1.4	5.3	29.7	36.4	61.8
.001 per cent. naphthaleneacetic acid + 0.5 per cent. Carbowax .	0.8	1.3	2.7	12.9	16.3	41.4
.001 per cent. 2,4 dichlorophenoxyacetic acid	1.9	5.3	10.7	18.1	19.4	24.9
.001 per cent. 4 chlorophenoxyacetic acid .	1.7	8.4	22.7	39.1	44.4	59.8
.001 per cent. 2,4,5 trichlorophenoxyacetic acid	1.7	8.4	21.0	39.9	45.9	64.6
Control	4.0	15.9	35.4	54.4	59.5	72.9
Difference necessary for significance at 5 per cent. point			7.7		11.8	11.0

DISCUSSION

In this experiment the addition of 0.5 per cent. Carbowax to naphthaleneacetic acid significantly increased both the intensity and the duration of effect. Results with the 2,4 dichlorophenoxyacetic acid treatment offer considerable promise as a means of extending the effective period of fruit-drop sprays. In order to obtain effectiveness with this compound comparable to naphthaleneacetic acid immediately following treatment, several possibilities suggest themselves: (1)

[1] F. E. Gardner, P. C. Marth and L. P. Batjer, SCIENCE, 90: 208–209, 1939.

using a spray solution stronger than 10 p.p.m.; (2) applying the spray at an earlier date with reference to fruit maturity; and (3) combining the 2,4 dichlorophenoxyacetic acid with Carbowax and with naphthaleneacetic acid, or with both.

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INFECTIOUS MYXOMATOSIS IN MALNOURISHED RABBITS

THOMPSON[1] and Parker[2] have demonstrated that infectious myxomatosis is modified in rabbits exposed to high external temperatures. In preliminary attempts to determine whether or not chemically induced fevers have a similar modifying action, we studied the action of dinitrophenol in 12 rabbits. Six of these rabbits were injected with myxoma virus and sustained at fever temperatures by the subcutaneous injection of alpha dinitrophenol (20 mg/Kg of a 3 per cent. sodium salt) twice daily at 12-hour intervals. Two of these rabbits (as seen in Table 1) showed a delayed and modified infection. Since these two rabbits had a distinct loss of weight we decided to investigate further the effect of malnutrition on infectious myxomatosis.

Twelve rabbits were kept on minimal amounts (5 to 20 grams) of stock rations for a period of 10 days prior to the injections of virus and during the course of the disease, except where premature death was expected. Myxoma virus was titrated intradermally[3] and temperatures, weights, tumor measurements and clinical signs were recorded daily. Thirteen tumor biopsies were made serially on starved and well-fed rabbits and examined microscopically to determine the progressive pathology.

Microscopic study of the tumor biopsies revealed that the process of tumor formation was retarded in the malnourished rabbits. Table 1 presents data on 9 malnourished rabbits; 3 others died before any significant observations could be made. Tumors in the malnourished rabbits were delayed in appearance, and were definitely smaller than those in the controls in this series or in any of the well-fed animals in our previous study.[3] In one malnourished rabbit only a single minimal lesion appeared in one of the 16 injected sites.

TABLE 1

INFECTIOUS MYXOMATOSIS IN MALNOURISHED RABBITS

Number of rabbits	Treatment	Weight loss in per cent.	Days after virus injection: Lacrimal discharge	Secondary lesions	Death	Gross pathology of tumors*
1	Malnourished	16	0	0	7	+
1	"	22	8	0	13	+
3	"	23–26	7–9	0	10–11	+++
1	"	28	8	8	11	++
1	"	29	0	0	8	+
1	"	30	8	0	10	+
1	"	34	0	0	8	+
4	Dinitrophenol	0	6–8	6–9	9–11	++++
1	"	10	9	0	9	+++
1	"	25	0	0	7	+
7	None	0	6–8	5–8	8–13	++++

* ++++ = typical, large, cyanotic, raised; +++ = delayed, typical but smaller; ++ = delayed, only slightly raised; + = small, not raised or edematous.

Strict comparisons of the 50 per cent. end-points could not be made because of the atypical appearance of many of the dermal lesions in the starved rabbits. The delay in the appearance of lacrimal discharges—or the complete absence of such—and the absence of secondary lesions in 8 of 9 rabbits suggests that there was less generalization of the virus than in the controls. The known mortality rate of about 100 per cent. and the duration of the infection were not modified. It should be noted that 4 of the starved rabbits died showing an atypical clinical picture of infectious myxomatosis.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS FOR GROWING MICROORGANISMS ON A FLOWING MEDIUM[1]

THE work of Shwartzman[2] describing the effect of Cellophane on penicillin production prompts us to publish the description of an apparatus used for growing microorganisms on cellulose tubing. We similarly used cellulose to increase the surface-volume ratio, but also to make a pathway through the growing fungus for the medium to pass into and out of the culture.

The apparatus (Fig. 1) consists of 50 feet of quarter-inch cellulose tubing[3] wound in a coil on

[1] R. L. Thompson, *Jour. Infect. Dis.*, 62: 307, 1938.
[2] R. F. Parker and R. L. Thompson, *Jour. Exper. Med.*, 75: 567, 1942.
[3] R. B. Houlihan and G. McL. Lawson, *Jour. Infect. Dis.* (in press).

[1] Journal Article No. 746 (n.s.) from the Michigan Agricultural Experiment Station.

[2] Gregory Shwartzman, SCIENCE, 100: 390, 1944.
[3] Blood transfusion tubing manufactured by Visking Corporation, Chicago, Illinois.

supports and suspended in a large Pyrex glass cylinder. The end of the tubing passes out through an aluminum cover held on the cylinder by means of metal strips passing underneath. Glass tubes are attached to the cellulose tubing where it passes through rubber stoppers in the aluminum top. This

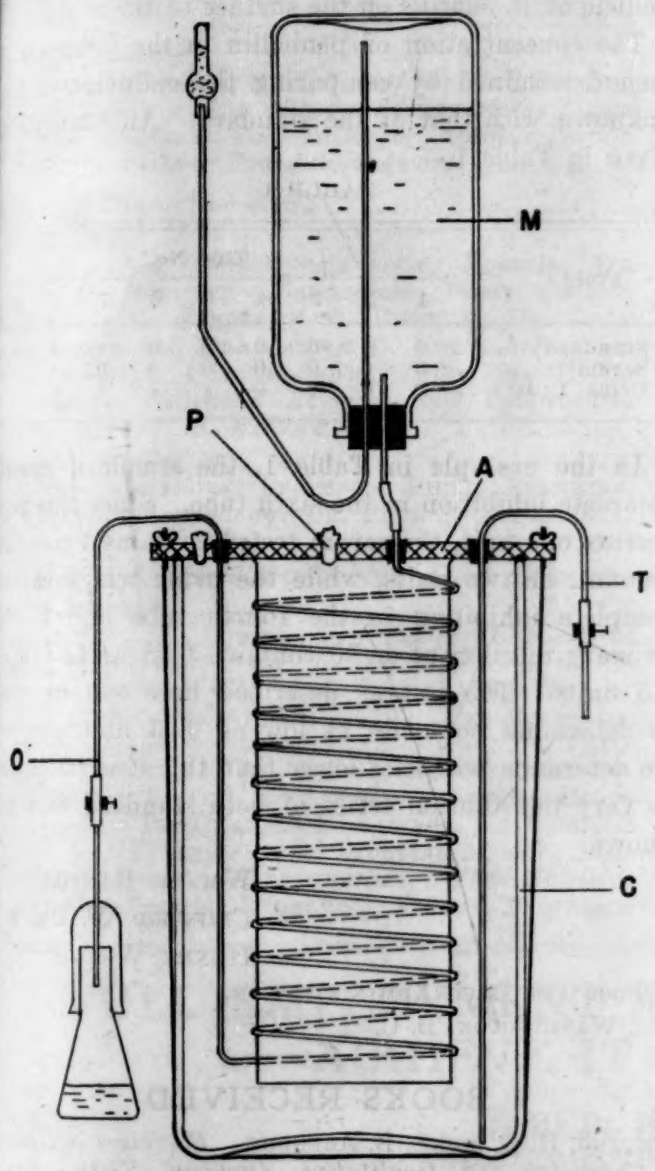

FIG. 1. M–medium. A–aluminum top. T–tube for removing seepage liquid. O–outlet tube. P–cotton plug. C-glass cylinder.

part of the apparatus is covered with a cloth hood, the outlets wrapped and sterilized in the autoclave.

A three-gallon bottle of sterile medium was prepared with an outlet assembly. It was then attached by a rubber tube to the cellulose tube inlet and supported so the medium would flow down into the cellulose tubing. The outside of the tubing was sprayed with a suspension of spores by reaching through the extra holes in the top. All necessary precautions were taken to prevent contamination.

A special atomizer was constructed by increasing the length of the two outlet pipes of a common DeVilbiss atomizer to 12 inches. A small Erlenmeyer flask was used in place of the regular fluid container. Sterile air was obtained by forcing air through water and then through a column of sterile cotton. The whole atomizer was constructed so that it could be autoclaved.

After inoculating with *Penicillium notatum*, the fungus grew readily and in two or three days the tubing was covered with mycelium. Usually the medium in the tubing was changed each day by draining through the outlet tube. The fungus continued rapid development and was covered with spores on the fourth or fifth day. When growth was well established, the medium was allowed to drop from the outlet tube at varying rates, usually about a liter per day. On the sixth to eighth day after inoculation, depending upon conditions, penicillin could be detected in the run-off medium. The penicillin titre increased within a few days so that it equaled or surpassed that of a flask culture. Unfortunately, the experiment could not be carried past the sixteenth day because leaks developed in the cellulose tubing owing to the action of the fungus.

An attempt was made to overcome the breakdown of the tubing by securing a thicker tube of cellulose and by the use of other materials. Thicker tubes of small diameter were not available. Cellulose nitrate films were found to be much more resistant to the fungus action. Attempts to coat the cellulose tubing with a cellulose nitrate film, either inside or outside, failed to give good results. Porcelain tubes with and without a coating of cellulose nitrate have so far failed to give the desired results, although they have overcome the problem of disintegration of the tubing.

Bacillus brevis was grown in this apparatus for three weeks without any apparent breakdown of the cellulose tubing.

The authors believe the apparatus as described can become a useful laboratory tool for studying certain organisms and that if a better tubing can be secured, it may be adaptable to the production of antibiotics and toxins.

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THE ESTIMATION OF PENICILLIN IN BODY FLUIDS

DURING the course of numerous investigations requiring the determination of penicillin activity in various body fluids, the need for a simple method of estimating penicillin levels of a lower order than that readily measured with accuracy by the agar cup plate method became apparent.[1]

A 4-hour turbidimetric assay employing *Staphylococcus aureus* was investigated but was found of little value with blood serums because of an apparent stimulation of the microorganisms by the presence

of the serum. The turbidimetric assay method, because of the erratic readings obtained, was abandoned in favor of the serial dilution method with a lengthened period of incubation.

Various methods for estimating the concentration of penicillin in body fluids have appeared in the recent literature. The method which seemed to possess the most merit was that of Rammelkamp,[1] or modifications thereof, in which a beta hemolytic streptococcus is employed in preference to staphylococci because of its greater sensitivity to penicillin. In order to increase the sensitivity, however, as well as the ease of determining the endpoint, washed red blood cells were used in the test. This necessitated establishing a source of washed cells and the use of young, freshly prepared cultures of the organism to prevent the addition of preformed lysin which interferes with the endpoint.

A large number of various species of bacteria were studied in an effort to determine whether an organism existed which was highly sensitive to penicillin yet could be grown and maintained on common laboratory media with a minimum of effort and which would give sharp and reproducible endpoints. The organism finally selected was a strain of *Bacillus subtilis*[2] obtained from the Northern Regional Research Laboratory. This organism grows luxuriantly at 30° C with a diffuse turbidity, while at 37° C growth consists of a pellicle with the medium below the pellicle remaining clear. Its resistance to penicillin is of the same order as most strains of hemolytic streptococci requiring as little as 0.0085 units per ml to inhibit a 1:100 dilution of a broth culture. This sensitivity is maintained over long periods of time without the necessity of repeated transfers. Cultures maintained at room temperature in screw-capped bottles were tested over an interval of six months with no demonstrable loss of sensitivity. Since then thousands of determinations have been made with satisfactory results.

Technique of the test: One-half ml amounts of broth[3] are placed in Wassermann-tubes and serial dilutions by halves made by adding one-half ml of the fluid being tested to one of the tubes and carrying one-half ml in serial dilution for as many tubes as necessary. The first tube in the series contains one-half ml of the material under test only. A standard is prepared for comparison by diluting a known potency penicillin (reference standard) to one-unit per ml in broth. This one-unit standard is diluted exactly as above in serial dilution by halves. One and one-half ml of a 1:100 dilution of the test organism in broth is then added and all tubes incubated at 37° C over night. The last tube in which no growth occurs is taken as the endpoint. This is usually very sharp, inasmuch as one tube will be absolutely clear while the next one in the series will have the typical pellicle of *B. subtilis* on the surface of the media.

The concentration of penicillin in the unknown is then determined by comparing the endpoint of the unknown with that of the standard. An example is given in Table 1.

TABLE 1

Fluid	Tube No.								
	1	2	3	4	5	6	7	8	9
Standard	0	0	0	0	0	0	+	+	+
Serum	0	0	0	0	0	0	0	+	+
Urine 1:10 ...	0	0	0	0	+	+	+	+	+

In the example in Table 1, the standard caused complete inhibition in the sixth tube. Since this represents one unit, the serum tested contains twice this amount, or two units, while the urine which caused complete inhibition in the fourth tube and had a primary dilution of 1:10 contains 0.25 units × 10 or 2.5 units. The test as described here can be used to determine potencies as low as 0.03 unit per ml. To determine potencies lower than this it is necessary to vary the dilution series of both standard and unknown.

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[1] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95, 1942.

[2] Some other strains of *B. subtilis* have been found to be considerably less sensitive.

[3] Mimeograph: Methods Used by the Food and Drug Administration for the Assay of Penicillin—Revised, January, 1945.

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